ASSESSMENT OF HEALTH, CONDITION AND THE EFFECTS OF PARASITES IN THE MOUNTAIN BRUSHTAIL POSSUM

A thesis submitted for the degree of Doctor of Philosophy of the Australian National University.

Karen Viggers (September 1996).

STATEMENT OF RESPONSIBILITY

The research reported in this thesis was conducted by me except where explicitly acknowledged.

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(September 1996).

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PREFACE

The trapping of *T. caninus* in this study was completed under the following permits:- Australian National University Ethics Committee (M.CRE.2.93; CRE.3.94; CRE.4.94; CRE.5.95 and CRE.6.96); Victorian Department of Conservation and Natural Resources (RP-92-116; RP-93-047; RP-93-146); State Forest of NSW (04716); NSW National Parks and Wildlife Service (Authority No. 1298); Queensland National Parks and Wildlife Service (1755) and Queensland Department of Primary Industries [Forest Service] (577; 1035/93; 31)

ABSTRACT

The main objectives of this study were to assess methods for evaluating health and condition in the mountain brushtail possum, *Trichosurus caninus* (Ogilby), and to examine the effects of parasites on the health and reproductive success of the species.

Reference haematological and serum biochemical values for *T. caninus* were established at a single study site at Cambarville in the central highlands of Victoria. Significant variation between sexes and between seasons was detected for some blood values. Values for haemoglobin concentration, red cell count and haematocrit were significantly higher in males than females. Total serum protein levels were higher in female *T. caninus*. Significant seasonal variation was detected for concentrations of total bilirubin, alkaline phosphatase, total protein, albumin, urea, absolute eosinophils, sodium, potassium and phosphate. There was no significant effect of reproductive status or excitability on blood values. Reference ranges of most blood variables exhibited wide ranges and considerable variability, which may limit their usefulness and sensitivity for detecting early stage, or subclinical, disease. Given this, other methods for quantifying or evaluating body condition were examined.

A condition index was derived for 104 *T. caninus* from seven study sites across the geographic range of the species, from linear regression of the relationship between body mass and body length. For *T. caninus*, body mass was related to the square of body length. This differed from geometric scaling theory, which suggests that body mass should be related to the cube of length. This suggests that if condition indices are to be used as measures of condition in animals, they should be derived independently for each species. Linear regression was used to examine variation in condition indices of *T. caninus*. Significant explanatory variables in the resulting statistical model were:- (**1**) the State in which the animal was trapped; condition index was higher in animals from Victoria than animals in NSW and Queensland, (**2**) faecal

egg count; condition index was negatively correlated with the log of the faecal egg count, (**3**) total serum protein; condition index was positively correlated with total serum protein levels, and, (**4**) the log of the absolute neutrophil count, which was also positively correlated with condition index.

The usefulness of condition indices depends on the assumption that they reflect body fat. This assumption was tested for *T. caninus* at Cambarville using dilution of isotopic water to estimate total body water composition. For many species, body water composition has been shown to be negatively correlated with body fat composition. For *T. caninus* at Cambarville, there was no significant relationship between condition index and body water composition, indicating that condition indices did not reflect body fat in this species. If animals with higher levels of body fat are recognised as being in better condition, then condition indices based on measures of body mass and size are not good indicators of body condition in *T. caninus*. Using this definition of body condition, estimates of total body water composition using equilibration of tritiated water are better for estimating body fat in *T. caninus*.

Having examined methods for assessing health and condition in *T. caninus*, the next part of the study aimed to examine the effects of parasites on the haematological and serum biochemical values of *T. caninus*, as well as on the reproductive success of this host. There was no detectable effect of prevalence or intensity of infection with most species of parasites on the haematological and serum biochemical values of *T. caninus*. This suggests that more sensitive methods are required to detect subclinical effects of parasites on the health of their hosts. However, host sample sizes were small, which may have reduced the chances of detecting subtle effects.

A systematic study of the helminths and ectoparasite species infecting *T. caninus* from seven study sites was completed to document the parasites infecting this host and their pathological effects. The ascaridoid nematode *Ophidascaris robertsi* and the metastrongyloid nematode *Marsupostrongylus minesi* caused pathological changes

to host tissues and may affect host health and fitness. Other potentially pathogenic parasites, such as the filarioid nematodes *Sprattia venacavincola* and *Brienlia trichosuri,* were recorded too infrequently to assess their effects on *T. caninus*.

Species richness of the parasite component communities of *T. caninus* was low in comparison with a number of other host species. This may be a consequence of the diet and foraging behaviour of *T. caninus*. No distinct patterns of co-occurrence were recorded between any helminth or ectoparasite species.

A field trial was conducted to confirm the efficacy of the anti-parasitic drugs ivermectin and praziquantel for removal or reduction in burdens of parasites infecting *T. caninus* at Cambarville. Ivermectin successfully removed parasites by ten days after treatment. Animals were re-colonised by parasites and shedding eggs in their faeces within eight weeks of treatment. Findings for the efficacy of praziquantel against the anoplocephalid cestode *Bertiella trichosuri* were inconclusive.

A field-based parasite burden manipulation experiment was conducted to investigate the effects of helminths and ectoparasites on the reproductive success of *T. caninus* at Cambarville. Adult female *T. caninus* in treatment and control groups were treated with ivermectin and praziquantel, or sham injected, at eight to ten week intervals throughout the breeding season. The proportion of females in the treatment and control groups that successfully reared young to emergence from the pouch was 0.69 and 0.60 respectively. This difference was not significant. No significant effect of removal of parasites from adult female *T. caninus* was detected on the birth, death or growth rates of pouch-dependent young. There was a significant effect of treatment with ivermectin and praziquantel on absolute eosinophil counts of adult females, which, in autumn, were higher in control females than in treatment females. However, there were no other significant effects of removal of parasites on the haematological and serum biochemical values of adult female *T. caninus*.

The results of the field experiment and parasite community study suggested that the parasite species infecting *T. caninus* at Cambarville were probably of low pathogenicity and were unlikely to regulate this host population. Potentially pathogenic parasites, such as *M. minesi* and *S. venacavincola* require further investigation to determine their effects on host health and fecundity.

TABLE OF CONTENTS

APPENDICES

LIST OF TABLES

LIST OF FIGURES

PLATES

CHAPTER 1

INTRODUCTION

An understanding of the mechanisms which influence population dynamics is required to assist in management decisions on the conservation of native wildlife. Parasites can have a major negative impact on their hosts by decreasing survival or reproductive rates, particularly under conditions of nutritional, social or hormonal stress. For example, high levels of mortality amongst juvenile eastern grey kangaroos, *Macropus giganteus*, in Australia, have been attributed to heavy burdens of a nematode parasite (Arundel *et al.* 1990). Diseases and parasites can also pose a significant threat to the survival of endangered species (van Riper *et al.* 1986; Thorne and Williams 1988; McCallum and Dobson 1995) or can jeopardise the success of conservation programs, such as reintroductions and translocations (Viggers *et al.* 1993). The importance of these effects, and the regulatory role of diseases and parasites on wild populations of animals, has been recognised by many authors (Anderson and May 1978, May and Anderson 1978; Anderson 1979; Holmes 1982; Keymer 1982; Scott 1988; Scott and Dobson 1989; McCallum and Singleton 1989; McCallum 1994, 1995; McCallum and Dobson 1995; Krebs 1995). In addition, recent interest in parasites as agents in the biological control of pest mammals (Spratt 1990; Singleton and Spratt 1986; Freeland 1993; Singleton *et al.* 1995; Singleton and Chambers 1996) indicates that further knowledge of their effects on wild populations is essential.

Parasites and diseases can affect hosts without playing a role in population regulation, but possibly influencing their evolutionary ecology (Holmes 1982, 1987, 1995; McCallum 1994, 1995). However, such effects are difficult to detect in free-ranging populations, as debilitated animals are rapidly removed by predators and

scavengers (McCallum 1994; McCallum and Dobson 1995). As a consequence, little is known of the disease status of wild populations, or of the effects of parasites on host health in many species of Australian native animals. Methods commonly used to assess health and condition, and to diagnose disease in domestic animals may be useful to detect and quantify some of these effects in native animal populations. Such methods include haematological, serum biochemical and serological analyses, as well as quantification of body condition. A better understanding of the impacts of parasites and diseases on their hosts could be gained by using these tools to assess health, in conjunction with information on parasite infracommunity structure and knowledge of the pathological effects associated with parasitic infection.

Many studies have investigated the impact of parasites on populations under laboratory conditions (Singleton and Spratt 1986; Spratt and Singleton 1986; Scott 1987, 1990; Atkinson *et al.* 1996). However, findings from studies on captive animals are often not applicable to wild populations, due to the major differences in many factors, including environmental and social conditions. There have been a number of investigations involving wild populations of animals (van Riper *et al.* 1986; Barker *et al.* 1991; Munger and Karasov 1991, 1994; Gulland and Fox 1992; Singleton *et al.* 1995; Singleton and Chambers 1996). However, there have been few attempts to manipulate parasite burdens in the field to study the role of parasites in limiting and regulating wild populations (but see Hudson *et al.* 1992; Gulland 1992; Lehmann 1992; Singleton and Chambers 1996). Manipulative field-based experiments are valuable as they allow hypotheses on the regulation of populations by parasites to be tested in the field (Krebs 1995; McCallum 1994, 1995; McCallum and Dobson 1995).

In this thesis, the mountain brushtail possum, *Trichosurus caninus* (Ogilby), was used as a case study to examine the effects of parasites on host health and condition, and on the reproductive success of adult female animals. Specifically this study aimed to:-

(1) establish reference haematological and serum biochemical values for *T. caninus* and examine variation in these values due to differences in sex, season, site, reproductive status, excitability and location of capture of animals (Chapter 4);

(2) evaluate the usefulness of blood values for detecting ill health in this species;

(3) examine methods for assessing and quantifying body condition of *T. caninus* in the field (Chapter 5);

(4) derive a condition index for *T. caninus* from linear regression of the relationship between body mass and a measure of skeletal size (Chapter 5);

(5) validate this condition index using dilution of isotopic water to determine whether condition index was correlated with body fat (Chapter 6);

(6) investigate serological evidence in *T. caninus* from seven populations, of exposure to a number of bacterial and viral diseases, to obtain baseline information on the status of these diseases in free-ranging populations of this species, and, to examine the potential for *T. caninus* to function as a reservoir for zoonotic diseases (Chapter 8);

(7) examine the variation in parasite infracommunities and component communities of *T. caninus* at seven study sites, to gain information on the distribution and occurrence of parasite species across the range of this host (Chapter 7);

(8) document the gross and histopathological changes associated with infection with various parasite species in this host, to determine which parasites may be more likely to have a negative effect on the host or host population (Chapter 8);

(9) examine the potential of parasite species infecting *T. caninus* for use in the biological control of the common brushtail possum, *Trichosurus vulpecula*, in New Zealand; and,

(10) determine whether parasites had a negative effect on the health and reproductive success on a wild population of *T. caninus* by completing a field-based parasite manipulation experiment, to examine the potential for host population regulation by the parasites infecting *T. caninus* at this site (Chapter 10).

In Chapter 2 a detailed discussion of previous research on the ecology, biology, haematology, serum biochemistry and parasites of *T. caninus* is presented.

Information on the biology and ecology of *T. caninus* was derived from studies of the species at three sites; one in northern NSW (How 1972, 1976, 1978, 1981) and two in central Victoria (Owen 1964; Owen and Thompson 1965; Seebeck *et al.* 1984; Lindenmayer *et al.* 1991a, 1996a, 1996b). This information was fundamental to understanding the population dynamics of the species, and designing the field study and field experiments.

Following the review chapter, the methods and procedures used in this project are described (Chapter 3). The remainder of the thesis is presented in two main parts. The first section (Chapters 4-6) focuses on the review and application of methods for assessing the health and condition of *T. caninus*. In the second part of the thesis (Chapters 7-10), the effects of parasites on *T. caninus*, in terms of clinical and subclinical health, and reproductive success, as well as the potential for parasites to regulate populations of *T. caninus* are examined. A general synthesis of the various components of the work is presented in Chapter 11. The potential usefulness of parasites of *T. caninus* for the biocontrol of the closely related common brushtail possum, *Trichosurus vulpecula,* in New Zealand, and some areas for future research, are also discussed.

CHAPTER 2

LITERATURE REVIEW

A REVIEW OF THE BIOLOGY, ECOLOGY, BLOOD CHEMISTRY AND PARASITES OF THE MOUNTAIN BRUSHTAIL POSSUM, *TRICHOSURUS CANINUS*, OGILBY (MARSUPIALIA: PHALANGERIDAE)

2.1 INTRODUCTION

The mountain brushtail possum, *Trichosurus caninus* Ogilby, is a nocturnal species of arboreal marsupial which inhabits tall wet open and closed forests and rainforests in eastern mainland Australia (Owen 1964; Owen and Thompson 1965; How 1972, 1976, 1978; Seebeck *et al.* 1984; Smith and Winter 1984; Lindenmayer *et al.* 1990). Although *T. caninus* is closely related to the common brushtail possum, *T. vulpecula*, populations of the two species rarely occur in sympatry (Owen 1964). *T. vulpecula* has a widespread distribution throughout the Australian mainland and Tasmania, whereas *T. caninus* has a more restricted range and appears to be confined to wet forests on the mainland (Troughton 1957; Owen 1964; Owen and Thompson 1965; How 1972, 1978; Lindenmayer *et al.* 1990).

This review outlines the ecology, population biology and reproductive strategies of *T. caninus*. In addition, information is described and summarised from previous studies on haematological and serum biochemical values of *T. caninus*, and parasites recorded from the species. Baseline data derived from previous research provided a foundation for the experimental design of studies in this thesis of health and disease in several wild populations of *T. caninus,* as well as the effects of parasites on the health and reproductive success of the species. Aspects of the biology and ecology of *T.*

vulpecula are discussed where they may enhance areas of limited knowledge for *T. caninus*.

2.2 TAXONOMY AND MORPHOLOGY

T. caninus belongs to the Family Phalangeridae. Members of this family include the genera *Trichosurus* (the brushtail possums), *Phalanger* (the cuscuses) and *Wyulda* (the monotypic scaly-tailed possum, *Wyulda squamicaudata*) (How 1991). The genus *Trichosurus* consists of two recognised species: *T. caninus*, the mountain brushtail possum, and *T. vulpecula*, the common brushtail possum. These species are generally separated on the basis of external morphology, including differences in body size, coat characteristics, prominence of the sternal gland, size and shape of the ear conch and skull size (Owen 1964; Owen and Thomson 1965; How 1972). *T. caninus* has small rounded ears, a clear sternal gland exudate and dense fur extending most of the way down the tail (Owen 1964; How 1972). In comparison, *T. vulpecula* is a smaller animal of slighter build, with larger, pointed ears, a more pointed face, less fur on the tail and a more prominent sternal gland with a darker exudate (How 1972). *T. caninus* is a larger, heavier animal, with a bodyweight range of 2.5-4.5kg, whereas *T. vulpecula* generally weighs between 1.7 - 2.4kg (up to 3.8kg in Tasmania) (Smith and Winter 1984). Both species have been hunted for their pelts to supply the international fur industry (Owen 1964). The value of *T. vulpecula* as a fur-bearing animal was the basis for its introduction to New Zealand in 1851 (Troughton 1957) where it has since attained pest status (Cowan 1990).

2.3 DISTRIBUTION

The distribution of *T. caninus* extends from Mt. Cole, just west of Melbourne (37°21'S, 143°15'E), Victoria (Anon. 1991; Victorian Mammal Database 1994), throughout eastern Australia and the Great Dividing Range to Kroombit Tops (24°22'S, 151°00'E) in central Queensland (see Fig 2.1). In the southern part of its range (central and eastern Victoria), the species is generally restricted to tall wet

sclerophyll forests, in regions with annual rainfall >1500 mm and occasional snowfalls (Owen 1964; Owen and Thomson 1965; How 1972; Seebeck *et al.* 1984; Lindenmayer *et al.* 1990, 1994a). Animals are locally abundant, particularly where the understorey is well developed (Owen 1964; Lindenmayer *et al.* 1990). Isolated populations of *T. caninus* also occur in the Dandenong (37°50'S, 145°15'E) and Strzelecki Ranges (38°19'S, 145°48'E) in Victoria (Anon. 1991). In the central and northern parts of its range, the occurrence of *T. caninus* tends to be patchy and disjunct. In New South Wales (NSW) and Queensland the species is generally found in subtropical rainforest, particularly in riparian areas, and in some coastal wet sclerophyll forest (Owen 1964; Smith and Winter 1984).

2.4 PREVIOUS STUDIES OF *T. CANINUS*

Previous field studies of *T. caninus* have been conducted in three places:- **(1)** Clouds Creek (30°04'S, 152°41'E) in northeastern NSW (How 1972, 1976, 1978, 1981; Barnett *et al.* 1979a, 1979b; Presidente *et al.* 1982); **(2)** Ben Cairn (37°43'S, 145°37'E), near Healesville in the central highlands of Victoria (Owen 1964), and, **(3)** Cambarville (37°34'S, 145°53'E), near Marysville in the central highlands of Victoria (Seebeck *et al.* 1984; Lindenmayer *et al.* 1990, 1991a, 1996a, 1996b).

A long term study of the ecology and reproductive biology of a natural population of *T. caninus* was completed by How (1972, 1976, 1981) at Clouds Creek. Barnett *et al.* (1979a, 1979b) completed the first haematological study of the species, whilst Presidente *et al.* (1982) examined the parasite faunas of *T. caninus* and *T. vulpecula* at this site. This population of *T. caninus* inhabited eucalypt forest that was progressively converted to pine plantation (How 1972; Barnett *et al.* 1976). Some individuals remained until all the native vegetation had been cleared and then finally disappeared (How 1972). The population of *T. caninus* at Clouds Creek no longer exists.

Owen (1964) completed a study of the ecology and home range of *T. caninus* at Ben Cairn, in Victoria. At Cambarville, Seebeck *et al.* (1984) and Claridge and Lindenmayer (1993) investigated the diet of *T. caninus*, and Lindenmayer *et al.* (1996a, 1996b) completed a major study of nest tree use by the species. In addition, Lindenmayer *et al.* (1990, 1994a) studied the habitat requirements of *T. caninus* in the central highlands region.

2.5 HABITAT REQUIREMENTS

Studies by Lindenmayer *et al.* (1990, 1994b) showed that a higher abundance of *T. caninus* was generally found at sites with greater numbers of hollow bearing trees, which provide nest sites for the species. In addition, the presence and abundance of the species was correlated with the density of *Acacia* spp. (Lindenmayer *et al.* 1990), the leaves of which are an important component of the diet of *T. caninus* (Seebeck, *et al.* 1984; Kavanagh 1984, 1987; Lindenmayer, *et al.* 1990) (see below).

Barnett *et al.* (1979a, 1979b, 1982) categorised two zones of habitat suitability for the purposes of their study on *T. caninus* and *T. vulpecula* at Clouds Creek in north-eastern NSW. "Preferred habitat" for *T. caninus* was defined as open and closed tall forest, whereas "peripheral habitat" was considered to be pine plantations, grazed woodland or open forests. *T. caninus* classified as being in peripheral habitat always included a portion of native open or closed forest within their range; this constituted a large part of the range of some animals (Barnett *et al.* 1979a). Therefore some of the animals categorised as being in peripheral habitat, may have been largely living in preferred habitat. These definitions of preferred and peripheral will be referred to throughout this thesis.

2.6 DIET

T. caninus is omnivorous and forages in both the understorey and on the ground (Owen and Thompson 1965; How 1972, 1978, 1981; Kavanagh 1984, 1987; Seebeck *et al.* 1984). At Cambarville, the foliage of *Acacia dealbata* was the preferred

food item of *T. caninus* (Seebeck *et al.* 1984). Leaf tissue and fungi were major dietary items throughout the year. Fungi were also eaten throughout the year, with peak consumption occurring in summer and autumn. In spring and early summer, floral parts, fruits and seeds occurred in the diet, reflecting their seasonal availability (Seebeck *et al.* 1984). A list of other items found frequently in the diet is presented in Table 2.1.

2.7 LONGEVITY AND SOCIAL STRUCTURE

T. caninus is long-lived and individuals of up to 10-17 years of age have been recorded both at Clouds Creek and Cambarville (How 1972; Barnett *et al.* 1982; Lindenmayer *et al.* 1991a). Female *T. caninus* generally live longer than males (How 1972).

Information on the social structure of populations can be provided by studies of home range use. Home ranges of *T. caninus* have been examined both at Clouds Creek (How 1972) and Ben Cairn (Owen 1964; Warneke *et al.* unpublished data). At Clouds Creek, adult male *T. caninus* had larger home ranges than sub-adult males and adult females, although extensive overlapping of home ranges occurred (How 1972). Each animal had a core area within its range where other animals were rarely trapped. Nearly all adult males and females shared a common core area, which was considered to be evidence for pair bonding and monogamy in the species (How 1972, 1981). At Ben Cairn, Owen (1964) found that *T. caninus* had very stable home ranges; those of males were larger than those of female animals. However, in contrast to Clouds Creek, there was little overlap of territories. Using trap-recapture data, Warneke *et al.* (unpublished data) found that the home ranges of male and female *T. caninus* at Cambarville were 3.98 and 3.78 ha respectively.

2.8 REPRODUCTION BIOLOGY

The breeding biology of *T. caninus* has received only limited attention in comparison to its congener *T. vulpecula*, which has been studied intensively in both Australia and New Zealand (Pilton and Sharman 1962; Smith *et al.* 1969). Detailed studies of the reproductive cycle of *T. caninus* have focussed on captive animals (Smith and How 1973) and the wild population at Clouds Creek (How 1972, 1976, 1981; Barnett *et al.* 1982).

T. caninus is monovular and polyoestrous (Smith and How 1973) and has a single autumn breeding season both in captivity and in free-ranging populations (Owen and Thomson 1965; How 1976; Barnett *et al.* 1982). The breeding season in wild *T. caninus* at Clouds Creek commenced in early autumn (February/March) and extended into early winter (May/July) (How 1972, 1976). Births were detected from February to May, with the majority of births occurring between mid March and the end of April. Some births took place after May, but these generally were second pregnancies of females which had lost young earlier in the season (How 1976). The breeding season was similar in central Victoria, with oestrus first occurring in March, and pouch young were detected from May to October (Owen 1964).

Female *T. caninus* attain sexual maturity in their third year (How 1972). This coincides with a change in pouch morphology, which converts from being a narrow fold of skin barely covering two inverted nipples, to a deeper structure (>3cm), with everted nipples (How 1976; Smith and How 1973). How (1972) observed that some wild females bred during their second year, but did not successfully rear young.

Normally, only one young is produced per year (Owen 1964). Females which lose pouch young may have a second young in spring, however there is a lower survival rate for young born later in the breeding season (How 1976). At Clouds Creek, females older than three years of age generally gave birth to young, but not all mature females successfully reared young every year (How 1972). There was a

gradual decline in fecundity with age, leaving an estimated 80% of adult females producing young (How 1978) (see Table 2.2). Males also reached maturity in their third year.

In wild populations of *T. caninus,* pouch young first release the teat approximately 112 days after birth. The young first emerge from the pouch at 175-200 days after birth (earliest observed: 155 days), and continue to suckle from outside the pouch until weaning after 240 days (How 1976). Emergent young initially are carried on their mother's backs until weaning between 240 and 270 days of age (How 1976). Young being carried on the mother's back are called "back young". From the time that young stop riding on the mother's back, until weaning, they are termed "juvenile", after which they are considered to be "sub-adults" until sexual maturity is attained.

Survival of young of *T. caninus* may be low. How (1976) observed that only 38% of young reached one year of age at Clouds Creek (compared with >85% survival in young of *T. vulpecula*). Most losses of young occurred during the pouch young stage, as only 44% of pouch young survived to the onset of weaning (How 1976). In central Victoria, Warneke *et al.* (unpublished data) recorded pouch young in 70% of females over three breeding seasons, however of 12 young produced, only two (17%) were known to survive to independence.

At Clouds Creek, pre-dispersal young remained in the maternal home range for some time and occasionally were caught in the same trap as the mother (How 1976, 1981). Dispersal in sub-adults occurred from 18 months of age. Males generally dispersed later than female animals and sometimes remained in the maternal territory for up to three years. Predation occurred predominantly on young that were emerging from the pouch and in dispersing sub-adults or those which had not successfully established a territory (How 1972). The known predators of *T. caninus* are: the powerful owl, *Ninox strenua*, the sooty owl, *Tyto tenebricosa*, the feral cat, *Felis catus,* the dingo, *Canis familaris,* the fox, *Vulpes vulpes* and the carpet python, *Morelia spilota variegata* (How 1981; Macfarlane 1988). Owen (1964) suggested that

the foraging behaviour of *T. caninus,* which includes considerable time foraging on the ground, may mean that it is susceptible to predation by *V. vulpes.*

2.9 HAEMATOLOGY AND SERUM BIOCHEMISTRY

Changes in haematological and serum biochemical values can be used as a clinical diagnostic and monitoring tool to detect organ dysfunction or disease (Bush 1991; Duncan *et al.* 1994). A wide range of tests may be performed to detect an alteration in the physiological status of an animal and identify the possible causes of that change. These tests include: counts and morphological assessment of blood cells, and measurement of levels of serum enzymes, metabolites and electrolyte concentrations (Bush 1991). Knowledge of the expected variation in blood profiles for a species is required to facilitate the interpretation of blood results and to enable inferences to be made about animal health and organ function. However, information on the reference blood values for many species of Australian native fauna is limited (Canfield *et al.* 1989a, 1989b).

There has been only one published study of the blood chemistry of *T. caninus*, in which the effects of age, sex, season and habitat on a number of blood values of the species at Clouds Creek were examined (Barnett *et al.* 1979a, 1979b). Blood characteristics measured by these authors were:- haematocrit, red cell count (RCC), haemoglobin concentration and plasma levels of glucose, protein and lipids.

There were significant differences between adult and sub-adult animals in a number of blood variables in *T. caninus* from Clouds Creek, including, plasma protein, glucose and haematocrit. Sub-adults had higher levels of plasma glucose than adult animals, whereas plasma protein levels were lower in sub-adults. In female animals, haematocrit was higher in sub-adult than in adult animals, however, this trend was not observed in male animals. The lower haematocrit in adult females may have resulted from female hormones (Barnett *et al.* 1979a), however this was not

confirmed. Haemoglobin concentration, haematocrit and RCC were consistently higher in male animals (Barnett *et al.* 1979a).

Significant variation between seasons was detected in RCC and haemoglobin concentration. The haemoglobin concentration was lowest in winter, whilst RCC was low in spring. There was also significant variation in haemoglobin values between successive years of the study, which may have been due to climatic variation. Plasma glucose levels were significantly lower in spring and summer, and highest at the end of winter, whereas plasma protein and lipid levels were found to be highest in summer.

The effect of reproductive status on the measured blood variables also was examined by Barnett *et al.* (1979b). Females with dependent or semi-dependent pouch young had significantly higher haematocrit values than those without pouch young. Females which had lost and not replaced pouch young had the lowest haematocrit. The authors suggested that these animals may not have been sufficiently healthy to successfully raise young (Barnett *et al.* 1979b), however the usefulness of haematocrit as a measure of health is not known for this species. Adult females showed an increase in plasma lipid levels while young were developing, which may have reflected a corresponding increase in milk lipids during lactation, as is seen in *T. vulpecula* (Gross and Bolliger 1959).

Animals from peripheral habitat at Clouds Creek had higher values for haematocrit, RCC, plasma protein and plasma lipids than those from preferred habitat. These differences may have been attributable to:- (**1**) variation in diet between habitats, although there was considerable overlap in the use of the two habitats, or, (**2**) variation in the population biology of animals from the two habitats (Barnett *et al.* 1979b).

There were no significant differences in the blood values of resident and dispersing *T. caninus*, which may have been due to the late dispersal of sub-adult *T. caninus* (Barnett *et al.* 1979b).

2.10 PARASITES RECORDED FROM *T. CANINUS*

There have been no systematic studies of the parasites of *T. caninus* across the geographic range of the species. The only detailed study of the parasite fauna occurring in a wild population of *T. caninus* was completed by Presidente *et al.* (1982) at Clouds Creek. These authors examined the effects of habitat, host sex and age on the parasites of *T. caninus*. The habitat definitions used were those established by Barnett *et al.* (1979a) for *T. caninus* at Clouds Creek (see 2.4 above). The prevalences of the oxyurid nematode *Adelonema trichosuri,* and third stage larvae of the ascaridoid nematode *Ophidascaris robertsi*, were higher in *T. caninus* in peripheral habitat than in those from preferred habitat. There was also an effect of habitat on the intensity of infection with some parasites: the intestinal nematode *Paraustrostrongylus trichosuri*, the ixodid tick, *Ixodes holocyclus* and the mites *Trichosurolaelaps crassipes* and *T. dixous*, were all present at higher intensities in animals from peripheral habitat. Sub-adult males had higher burdens of *Paraustrostrongylus trichosuri* than sub-adult females.

Other parasite records from *T. caninus* have been based mainly on the capture of small numbers of animals in studies encompassing a range of host taxa (see Viggers and Spratt 1995, Appendix 1). A detailed description of the helminths and arthropod ectoparasites known to occur in *T. caninus* and their associated pathological changes is given in Appendix 1 (Viggers and Spratt 1995). Information on the effects of these parasites on host health and reproduction may be applicable to biological control programs for *T. vulpecula* in New Zealand.

Table 2.1: Dietary items of *T. caninus* at Cambarville, central Victoria (adapted from Seebeck *et al.* 1984)

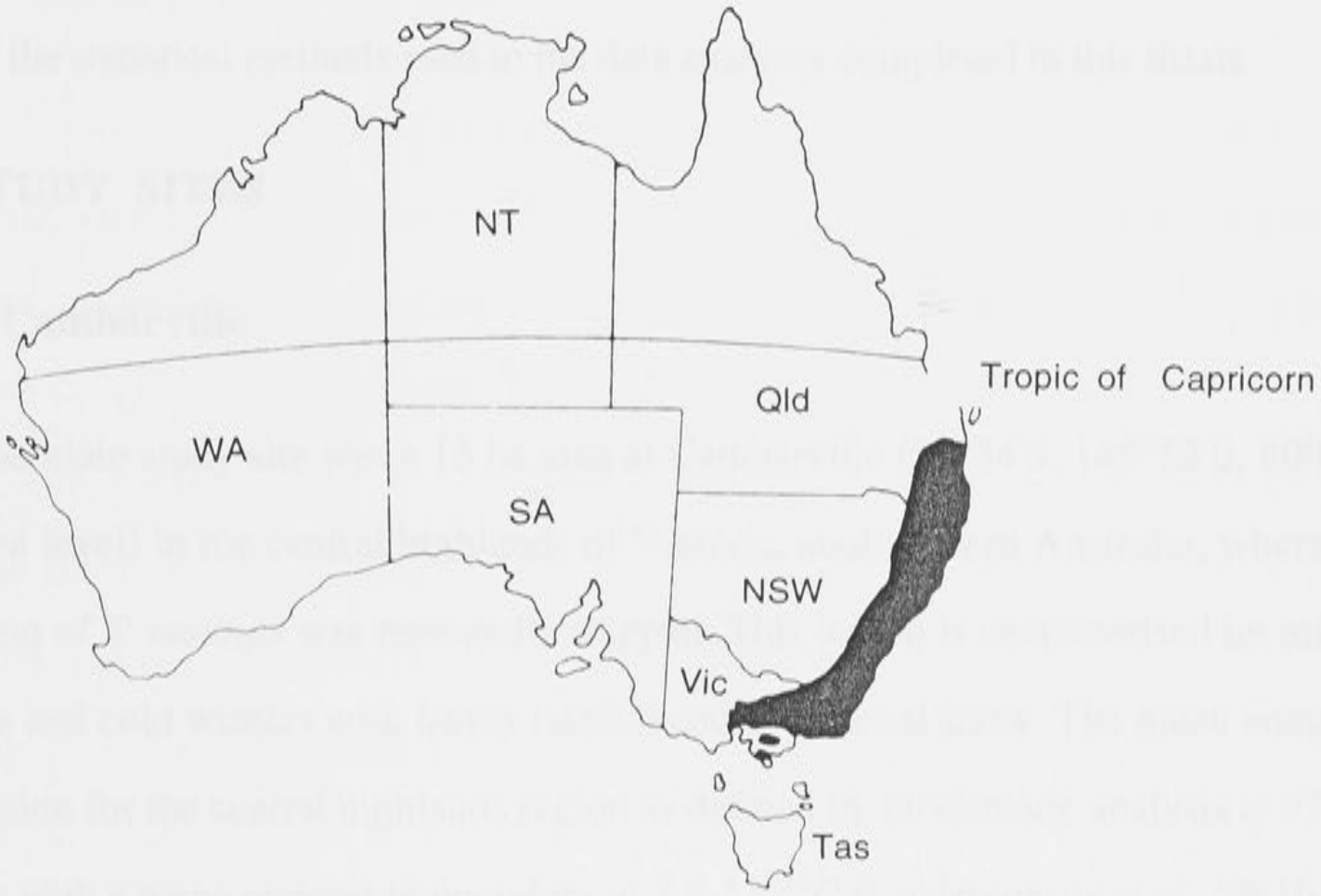
Dietary Item	Species
Leaf tissue	Silver Wattle *(Acacia dealbata)*
	Tree ferns, *(Dicksonia antarctica, Cyathea australis)*
	Victorian Christmas Bush *(Prostanthera lasianthos)*
	Blackberry *(Rubus fruticosus* spp.)
	Bidgee-Widgee *(Acaena anserinifolia)*
	Mountain Pepper *(Tasmannia lanceolata)*
	Myrtle Beech *(Nothofagus cunninghamii)*
	Montane Wattle *(Acacia frigescens)*
	Hazel Pomaderris *(Pomaderris aspera)*
Fungi	
Pinus radiata staminate cones	
Floral parts	
Arthropods, grass, seeds, moss	

Table 2.2: Age specific fecundity for known age and assessed age *T. caninus* at Clouds Creek in northeastern NSW (adapted from How 1972).

	Age (years)					
	1	2	3	4	5	6
No. females	10	9	14	11	8	4
No. breeding females	0	1	14	9	7	3
No. young	0	5	14	12	7	3
Fecundity*	0	0.56	1.0	1.1	0.88	0.75

* fecundity was determined by dividing the number of young by the number of females

Figure 2.1: The distribution of the mountain brushtail possum, *Trichosurus caninus* (Ogilby) (adapted from Strahan 1991; limits of the distribution are approximate)



Vic = Victoria
NSW = New South Wales
Qld = Queensland
NT = Northern Territory
SA = South Australia
WA = Western Australia
Tas = Tasmania

CHAPTER 3

STUDY SITE DESCRIPTIONS AND GENERAL METHODS

In this project, *T. caninus* was studied at seven sites across the geographic range of the species. These sites are described below. Several key methods and techniques were used in the collection and processing of samples from *T. caninus* in the field. These methods are also outlined in this chapter. In addition, a brief outline is given of the statistical methods used in the data analyses completed in this thesis.

3.1 STUDY SITES

3.1.1 Cambarville

The main study site was a 15 ha area at Cambarville (37°34'S, 145°53'E, 800m above sea level) in the central highlands of Victoria, southeastern Australia, where a population of *T. caninus* was repeatedly trapped. This region is characterised by mild summers and cold winters with heavy rainfall and occasional snow. The mean annual precipitation for the central highlands region as defined by bioclimatic analysis is 975-1800mm with a mean average temperature of 7.8-13.4°C (Lindenmayer *et al.* 1991b).

Cambarville is situated in the Cumberland Scenic Reserve within the Yarra Ranges National Park. The site was a former logging settlement established to mill logs from trees which were damaged by wildfires in 1939. The timber mill at Cambarville was closed in 1970 (Seebeck *et al.* 1984). Cambarville consists of a clearing amidst tall wet eucalypt forest dominated by mountain ash, *Eucalyptus regnans*. On the eastern side of the clearing is a small pine plantation (~1ha) in which some *T. caninus* were regularly trapped. However, most of the forest covering the 15 ha study site consists of *E. regnans* which exceed 300 years of age except an area in the northwestern corner of the site, which regenerated after the 1939 fires.

Several wildlife studies have been completed at this site, including studies of the endangered Leadbeater's Possum, *Gymnobelideus leadbeateri*, (Smith 1980, 1984, Lindenmayer 1991c; Lindenmayer and Meggs 1996) and *T. caninus*, (Seebeck *et al.* 1984; Lindenmayer *et al.* 1991a, 1996a, 1996b; Claridge and Lindenmayer 1993). A high density of *T. caninus* exists at Cambarville (1.1 animals per hectare; Lindenmayer, unpublished data), which may be associated with the large number of potential nest trees and the presence of suitable ground and understorey plant species for foraging (Lindenmayer *et al.* 1990; Chapter 2.5).

3.1.2 Comparative study sites

T. caninus was trapped at an additional six sites in eastern Australia (see Fig 3.1). These were:- (**1**) Bellbird (37°39'S, 148°50'E, 330m above sea level), East Gippsland, eastern Victoria, (**2**) Allyn River State Forest (32°07'S, 151°28'E, 300m above sea level), near Barrington Tops National Park, central NSW, (**3**) Whian Whian State Forest (28°87'S, 153°20'E, 400m above sea level), near Lismore, northeastern NSW, (**4**) Byrangery Reserve (28°87'S, 153°25'E, 200m above sea level), near Lismore, northeastern NSW, (**5**) Conondale Ranges (26°26'S, 152°35'E, 400m above sea level), southeastern Queensland, and, (**6**) Bulburin State Forest (24°33'S, 151° 28'E, 400m above sea level), central Queensland. These sites were selected because they were distributed across the geographic range of *T. caninus*, and because the species was known to occur at reasonable densities in these areas.

The Bellbird study site was dominated by tall wet sclerophyll forest. Cage traps have been regularly set at this site by the Victorian Department of Conservation and Natural Resources as part of an ongoing study of the long-footed potoroo, *Potorous longipes* (Scotts and Seebeck 1989). Individuals of *T. caninus* often have been trapped as a non-target species in that study. The site is situated on a steep slope, which was burned for fuel reduction in 1974. In 1975, the remaining large trees on the ridges and lower slopes were ring-barked and poisoned to enhance regeneration

(Scotts and Seebeck 1989). Consequently, there are numerous trees with hollows which provide nest sites for *T. caninus* on the site.

At Barrington Tops, animals were trapped in wet sclerophyll forest within 40m either side of the Allyn River. In Whian Whian State Forest, *T. caninus* was mainly trapped in areas of regenerating rainforest with a dense understorey, and often along streams or drainage lines. In contrast, at Byrangery Reserve, within 15 kilometres of Whian Whian, the species was trapped in gullies embedded in farming land where rainforest was in early stages of regeneration.

In the Conondale Ranges, animals were trapped almost exclusively along streams, often in disturbed areas within sub-tropical rainforest and wet sclerophyll forest. At Bulburin State Forest, animals were captured in sub-tropical rainforest along drainage lines or intermittent stream beds, and were also trapped around the edges of a clearing, where the rainforest canopy was dense.

3.2 TRAPPING AND HANDLING PROCEDURES

3.2.1 Trapping methods

T. caninus was trapped in Mascot wire cage traps (600 x 300 x 300 mm) baited with apple. Traps were either a treadle design or had a hook mechanism which is released when the animal grasps the bait. Knowledge of the habitat requirements of the species was important for identification of places where trapping was likely to be successful (see Chapter 2.5). Traps were placed on the ground near large hollow trees or on fallen logs. At Cambarville, the same sequence of trapping at identical trap placement points was used during each trapping period. At some sites (southeastern and central Queensland), where *T. caninus* appears to spend less time feeding on the ground, aniseed oil was applied to the apple as a lure to attract possums to the ground to facilitate trapping. This technique has been used successfully in New Zealand for the capture of *T. vulpecula* (Efford, pers.comm.). Traps were set in the afternoon and were checked early the next morning.

To minimise the stress of capture, each animal that was trapped was transferred to an individually labelled hessian sack. Prior to this, a subjective rating was made of the excitability of each animal in the trap. These scores ranged from one (quiet) to five (highly excitable). Animals were sedated to facilitate physical examination and the collection of blood samples (see below and Appendix 2: Viggers and Lindenmayer 1995). Whilst under sedation, animals were weighed, measured, assigned to an age-class (see below), and a 3ml blood sample was collected from the jugular vein, using a 3ml syringe and 25g needle. Animals trapped at Cambarville were tattooed in the pinna of the ear for future identification. The pouch of each female was checked to determine reproductive status. Heart rate, respiratory rate and rectal temperature of each animal were recorded. Finally, a range of morphological body length measurements (Table 3.1) was collected for each animal using Vernier calipers (to the nearest 0.1mm) or a standard tape measure (to the nearest 0.5cm).

3.2.2 Trapping effort

During each trapping session at Cambarville throughout this study, 60 traps were set each night for ten nights (ie. 600 trap nights). At the other study sites, 50 traps were set each night for seven nights (ie. 350 trap nights). Setting of traps required considerable effort carrying traps through heavy scrub and understorey, sometimes for long distances. Clearing and re-setting of traps each morning usually took from two to four hours depending on the number of animals captured.

3.3 SEDATION AND OTHER DRUG USE

T. caninus were sedated with tiletamine/zolazepam (Zoletil 100®) (Virbac, Sydney, Australia) by injection into either the gluteal muscles or the muscles of the cranial thigh (see Appendix 2: Viggers and Lindenmayer 1995). A standard total dose of 50 to 60 mg per animal was used. If sedation was inadequate, additional smaller doses of tiletamine/zolazepam were given. Sedation was better in those animals given a single dose. Animals that began to recover before completion of handling procedures

were easily restrained manually until all procedures were completed. After recovery from sedation, animals were released at the point of capture.

As part of a field-based parasite manipulation experiment (see Chapters 9 and 10), some animals were injected subcutaneously with ivermectin (Ivomec®) (MSD Agvet, Sydney, Australia) (@200µg/kg) and praziquantel (Droncit ®) (Bayer, Sydney, Australia) (@10mg/kg) using separate syringes for each drug.

3.4 AGE DETERMINATION

Age class of *T. caninus* was estimated by subjective assessment of the wear of the upper left first molar (see Fig 3.2). This method was developed by Winter (1980) in a study of *T. vulpecula*. Although wear classes do not necessarily correspond with years of age (see Winter 1980), this method allowed a structure of age classes within a live population of animals to be established. There are two main sources of error in this method:- **(1)** variation in wear due to dietary differences between sites, and, **(2)** human variation in evaluation of changes associated with wear. It was assumed that within a study site there would be only limited variation in available dietary items, thus minimising variation between individuals associated with differences in the diet (Winter 1980). To overcome the problem of error between observers, the same observer (KLV) completed all age assessments.

Although age in years could not be determined from tooth wear, this method for assessing age was considered to be useful. For *T vulpecula*, tooth wear classes corresponding to ages in years were as follows: up to one year, wear class 1; one to two years, wear classes 1,2; three years, wear classes 2,3; four years, wear classes 3,4,5; five years, wear classes 4,5,6; six years, wear classes 6,7,8,9; seven years wear classes 5,6,7,9, eight and nine years, wear classes 7,8,9 (Winter 1980).

In this thesis, juvenile and sub-adult *T. caninus* displayed tooth wear categories in age classes one and two. All mature animals had tooth wear corresponding to age classes three or greater.

3.5 HAEMATOLOGICAL ANALYSES

From each animal, a total of 1ml of blood was transferred into a blood tube containing ethylenediaminetetra-acetic acid (EDTA). Samples from Cambarville animals that were collected in the first year of the study (1992-1993) were stored at 4°C and then forwarded on ice to Dorevitch Pathology of Camberwell, Victoria. Haematological analyses were completed within 16 hours of blood collection using a Sysmex NE-8000 Automated Haematological Analyser (JOA Medical Electronics Co Ltd., Kobe, Japan) adjusted for cell size (see Whittington and Comer 1984). Haematocrit was determined by the microhaematocrit method (Schalm 1975). After the first year of the study, all haematological analyses, except haemoglobin measurement, were performed in the field. The haematological parameters that were measured are given in Table 3.2.

Red and white cell counts were determined using the Unopette® system (Becton-Dickinson, c/o Bactolabs, Sydney) and a Neubauer Improved Haemacytometer. For white cell counts (WCC), 20µl of whole blood was added to 1.98ml of diluent (3% acetic acid) to haemolyse mature red blood cells. The diluted blood was added to the haemocytometer and the number of cells in all of the nine large squares of the counting chamber (Fig 3.3a) was counted under X100 magnification. The WCC (in cells/mm^3) was obtained by adding 10% of the count to the total number of cells and multiplying by 100. To convert to standard units (x 10^9/l), WCC per mm^3 was divided by 1000. If nucleated red blood cells were detected in the blood smear (see below), the WCC was adjusted as follows:

$$\frac{\text{No. nucleated RBC}}{100 + \text{No. nucleated RBC}} \times \text{WCC/mm}^3 = \text{absolute no. of nucleated RBC/mm}^3$$

$$\text{Corrected WCC} = \text{WCC/mm}^3 - \text{nucleated RBC/mm}^3$$

To determine RCC, a total of 10µl of whole blood was added to 1.99ml of diluent (isotonic saline). This diluent retards lysis and crenation of red blood cells and preserves white cells. Diluted blood was added to the haemocytometer and X400 magnification was used for counting. Red blood cells were counted in the four corner squares and the centre square within the large centre square of the counting chamber (see Fig 3.3b). The total number of cells in the five squares was multiplied by 10,000 to give the number of red cells per mm^3. To convert to current standard units ($x10^{12}/l$), RCC per mm^3 was divided by six.

Thin smears were made within one hour of collection from the blood stored in EDTA. Smears were fixed in methanol and stained with Giemsa. Differential white cell counts were performed at X1000 magnification by differentiating 200 white cells in the monolayer part of the smear by means of a battlement method (Fig 3.4; Benjamin 1978). Smears were also examined for the presence of blood parasites and to assess white and red blood cell morphology. The percentages of neutrophils, band neutrophils, lymphocytes, monocytes, eosinophils and basophils were determined and absolute values for each cell type were calculated as follows (Bush 1991):

$$\text{Absolute count of WBC type (in } 10^9/l) = \frac{\text{differential count of that WBC type (\%)}}{100} \times \text{total WCC } (10^9/l)$$

3.6 SERUM BIOCHEMICAL ANALYSES

From each animal, 1.5-2ml of blood was transferred into a plain serum tube. After clotting had taken place (2-3 hours), the blood was centrifuged at 3000 rpm for ten minutes to separate the serum from the clot. The resulting serum was stored and transported as for EDTA blood (see above). Biochemical analyses were performed within 16 hours of blood collection by Dorevitch Pathology using a Kodak Ektachem E 700 Automated Biochemistry Analyser (Johnson and Johnson Clinical Diagnostics,

Rochester, New York). When it was not possible to submit samples within 16 hours, they were stored in liquid nitrogen and then transported on dry ice to Dorevitch Pathology. The serum biochemical tests that were completed are shown in Table 3.3.

3.7 COLLECTION OF FAECAL SAMPLES AND ECTOPARASITES

Faecal pellets were collected from the bottom of the trap in which each animal was captured and preserved at the time of collection in 10% formalin for faecal egg counts. Ectoparasites were collected from sedated adult animals. Fine forceps were used to remove ticks, taking care to remove mouth parts for subsequent identification purposes. To collect fleas and mites, each sedated *T. caninus* was placed in a large plastic bag, with the head outside. A small glass bottle containing cotton wool soaked in chloroform was placed inside the bag and rubbed over the fur. Ectoparasites in the pelage were removed by vigorous brushing of the animal over white cartridge paper. Mites and fleas were then collected and stored in 70% ethanol. Dirt and debris remaining on the cartridge paper were swept into ethanol, as this often contained small mites that were not easily visible. Ectoparasites were identified and counted upon return to the laboratory. Ectoparasites were not collected from female *T. caninus* carrying pouch or back young because of the risk of killing the young by exposure to chloroform.

3.8 FAECAL ANALYSIS

3.8.1 Faecal flotation

Faecal egg counts may provide an approximate measure of the intensity of parasitic infection in living individuals (Anderson and May 1991). Using faecal samples from each *T. caninus* that was captured, a faecal flotation (Thienpont *et al.* 1979) was completed using a Universal slide, which contains two counting chambers, each with a capacity of 0.5 ml. A total of 10g of faeces was mixed with 20ml of faecal flotation fluid (saturated sodium nitrate: 616g/l) and a stirring rod was used to break down the pellets. Faecal flotation fluid was then added to bring the total volume to

60ml. This was sieved through a fine tea-strainer to remove larger vegetative debris. The resulting suspension was mixed thoroughly and a small aliquot immediately transferred to fill the two counting chambers. The slide was examined under a light microscope and all parasite eggs in both chambers were counted. Coccidial oocysts were also recorded and counted. The total number of eggs or oocysts per gram was calculated by multiplying the total eggs or oocysts counted in the two chambers by a dilution factor of 66.

3.8.2 Faecal analysis for *Bertiella trichosuri*

The eggs of some anoplocephalid cestodes may float in saturated sucrose or sodium nitrate (eg. Proudman and Edwards 1991). However, eggs containing embryos of *Bertiella trichosuri* in the faeces of *T. caninus* appeared to be too heavy to be suspended using the saturated sodium nitrate in the faecal flotation technique. Hundreds of faecal flotation examinations were completed in this study, and no eggs of *B. trichosuri* were detected, even in faeces of necropsied animals that were known to be infected with this parasite. Given this, the method of Knapp and Presidente (1971) for recovering *Fasciola hepatica* ova was attempted to detect eggs of *B. trichosuri*. A total of 10g of faeces was washed over two Tyler sieves (200µm and 50µm for the upper and lower sieves respectively). The material collected on the 50µm sieve was washed into a sedimentation flask and allowed to stand for 30 minutes. The supernatant was discarded, the debris in the bottom of the flask resuspended and then allowed to settle once again. The supernatant was again discarded and the sediment poured into a lined petri dish for counting under a dissection microscope at X16 magnification. This magnification was not adequate for easy detection of *Bertiella trichosuri* eggs, which are not a readily visible tan colour like those of *F. hepatica*. Even under higher magnifications, the eggs of *B. trichosuri* were very difficult to distinguish from vegetative debris of similar size. On this basis, the technique was considered not to be useful for this work. Another method whereby eggs can be easily identified from debris is required.

3.8.3 Baermann technique for the collection of lungworm larvae

Lungworm larvae were collected using the Baermann technique (Thienpont *et al.* 1979). A small plastic funnel connected to a clamped length of rubber tube was attached to a stand (Fig 3.5). Fresh macerated faeces were placed in gauze in the top of the funnel and warm water was added to cover the gauze and faeces. This was left to stand for 30 minutes for any metastrongyle larvae present to migrate out of the faeces and descend to the clamped tube. The clamp was then released and a small volume of fluid collected in a centrifuge tube. This liquid was spun in a centrifuge at low speed, the sediment collected from the bottom of the tube and examined under a light microscope for the presence of larvae.

This technique was used in the first year of the study for *T. caninus* at Cambarville. However, the use of Baermann tests was discontinued due to repeated collection of hatched larvae from eggs of gastrointestinal nematodes that were also present in the faeces. This was probably due to the delay between passing of faeces by animals and completion of the Baermanns tests in the laboratory, during which hatching of intestinal nematode eggs occurred.

3.9 CONCENTRATION TECHNIQUE FOR DETECTION OF MICROFILARIAE IN BLOOD

Female worms of *Sprattia venacavincola* and *Breinlia trichosuri* produce microfilariae which circulate in the peripheral blood (Spratt and Varughese 1975). Microfilariae were detected using the method of Whitlock *et al.* (1978) which was developed to detect microfilariae of *Dirofilaria immitis* in blood. From each *T. caninus*, a total of 1ml of blood was transferred into a blood tube containing EDTA to prevent clotting. A lysing solution was prepared: 8g of sodium carbonate per litre of distilled water + 0.5% formalin. The blood was drawn up into a 10 ml syringe containing 9ml of lysing solution and mixed well by inverting the syringe several times to lyse the red blood cells. The resulting solution was passed through a

polycarbonate filter (5µm), which was assembled in a filter holder. The filter was flushed with 10ml of distilled water and then 10ml of air to dry the filter membrane. The filter paper was placed onto a microscope slide and stained for 30 seconds with a few drops of methyl green. A coverslip was applied and the slide was examined under a light microscope at X100 magnification for the presence of microfilariae. Microfilariae were identified according to the descriptions of Spratt and Varughese (1975). Microfilariae of *S. venacavincola* are sheathed (Spratt and Varughese 1975), whereas those of *B. trichosuri* are unsheathed (Breinl 1913; Spratt and Varughese 1975), and these features are readily identifiable under X400 magnification.

3.10 POST MORTEM EXAMINATION

3.10.1 Sample sizes

Post mortem examinations were performed on a sub-sample of animals to enable a detailed investigation of helminth parasites (see Chapter 7) and to collect tissues for histopathological examination (see below). Sample sizes of *T. caninus* removed from most sites were small (1-4) due to difficulties in obtaining permits to collect and euthanase animals, given that native animals are protected in Australia. Collection of animals from the trapping grid at Bellbird in east Gippsland was not permitted due to ongoing studies of *Potorous longipes* at that site. Consequently, a considerable amount of time was spent (17 days over two field trips) attempting to trap animals in the adjacent region. Despite a concerted trapping effort (50 traps set per night) only one animal was captured for post mortem examination.

3.10.2 Post mortem procedure

Prior to euthanasia and post mortem, each animal was sedated using tiletamine/zolazepam for blood and ectoparasite collection. Whilst under sedation animals were euthanased by lethal intracardiac injection of pentobarbitone.

The animal was placed in a large white tray for the post mortem examination. It was then systematically skinned and the subcutis was examined for the presence of nematodes. A ventral incision was made either side of the mandible to allow the tongue to be removed from the oral cavity. The tongue, trachea and oesophagus were then dissected down to the thoracic inlet. The abdominal cavity was entered by a ventral midline incision and the peritoneal fluid and mesenteries were examined for the presence of filarioid nematodes and ascaridoid larvae. All abdominal organs were examined for gross pathological abnormalities. The gastrointestinal tract was tied off at the rectum and the oesophagus, then removed in its entirety, with the liver and spleen attached and placed in 0.9% saline (sodium chloride) to prevent desiccation. The kidneys and reproductive tracts also were removed.

Penetration of the diaphragm allowed access to the thoracic cavity. The lungs and heart were examined *in situ* for the presence of gross pathological lesions, then removed in conjunction with the oesophagus, trachea and tongue and placed in 0.9% saline. Dissection of the cranium was performed to remove the brain. All organs were weighed prior to sectioning. Pieces of tissue from organs (see Table 3.4) and any grossly visible lesions were preserved in 10% formalin for later histopathological examination.

Examination of the lungs for the presence of metastrongyloid nematodes (lungworms) was performed under a dissecting microscope using transmitted light. Small tissue sections were pressed between two glass petri dishes to enable the observation of the parasites within the bronchioles and pulmonary parenchyma. Parasites were carefully dissected out of the lung tissue, fixed in hot 10% neutral buffered formalin for five minutes and preserved in 70% ethanol for later identification. Parasites which were deeply embedded in lung tissue and could not be easily removed were preserved within the lung tissue in 2% formalin and subsequently removed in the laboratory.

Examination of other organs and inspection of the gastrointestinal tract for pathological lesions and helminth parasites was then undertaken. For ease of examination, the small intestine was sectioned into 10cm lengths. Each portion was opened longitudinally and the contents were collected in a lined petri dish. If any cestodes were present that could not be easily removed, the section of intestine was soaked in tap water for up to one hour, during which time the cestode relaxed and could be easily removed. The mucosa of the intestine was scraped with a pair of blunt, flat forceps to remove any helminths coiled around the intestinal villi or resident in the mucous layer. After the addition of saline, the sample was examined under a dissecting microscope with incident light. A subsample of each parasite species detected was set aside in 0.9% saline. Each species of parasite was counted. To ensure that no parasites were missed, each section of gut was also examined using the "press" technique described for lungworm detection (see above).

The caecum generally contained a large quantity of semifibrous material. This was transferred to a petri dish in small portions, saline was added and then teased apart with forceps to check for the presence of oxyurid nematodes. If parasites were extremely numerous, all of the caecal material was diluted with a measured quantity of saline to bring the total volume to 1000ml as described by Clark *et al.* (1971). This was mixed well and four subsamples each of 10ml were taken to enable parasites to be counted. The dilution factor could then be calculated and a parasite count for the entire caecal matter thus determined. The number of counts of 1% aliquots to be completed to ensure a 5% standard deviation between counts was based on the number of worms present in the initial four subsamples (see Clark *et al.* 1971).

Liver, kidneys and spleen were teased apart and carefully dissected with fine scissors and forceps. The gall bladder, bile ducts and major blood vessels were incised and examined under a dissecting microscope at X16 magnification for the presence of helminths.

Initially, all parasites were collected into 0.9% saline. Nematodes were then preserved in boiling 10% neutral buffered formalin for five minutes, and left to stand for 12 hours, before being transferred into 70% ethanol for storage. Cestodes were relaxed in water, then fixed in 10% neutral buffered formalin. Parasite identification was performed in the laboratory after the completion of each field trip.

3.11 HISTOPATHOLOGICAL EXAMINATION

Tissue samples from most organs and any gross lesions were fixed in 10% neutral buffered formalin for histopathological examination. Samples were collected as soon as possible after euthanasia of the animal. Pieces of small intestine were collected before scraping of the mucosa to remove any parasites embedded in the mucosa (as described in 3.10 above). Trimmed tissues were embedded in paraffin and sectioned at 6μm. All sections were stained with haematoxylin and eosin and mounted on microscope slides for examination. All slides were examined initially at 40X magnification for anomalies in tissue structure and architecture, and obvious lesions. Unusual or abnormal areas were examined at higher magnifications.

3.12 STATISTICAL METHODS

Statistical models describing relationships between response and explanatory variables provide a concise summary of data, as well as a basis for inference. In this study, a number of statistical methods were used for the analysis of data, including ordinary linear regression (Weisberg 1980), logistic regression (Collett 1991), Poisson regression (McCullagh and Neldner 1989) and principal coordinates analysis (Krzanowski 1988). For data with a hierarchical structure, mixed models were used (Engel 1990). In this section, each statistical method used in this thesis is briefly outlined and an explanation of the choice of methods is given. A description of the process of modelling and model selection is also presented.

3.12.1 The modelling process

In general, data summarised in the form of a statistical model consist of two components, a mean function (ie. the mean relationship) and a variance function (ie. error). In all cases, the choice of statistical model must be based on the question being asked, the structure of the data and the distributional properties of the response. The most parsimonious model which explains most of the variation within the data is selected. The process of analysis of data involves several stages, including:- (**1**) exploratory data analysis (eg. graphical analysis), (**2**) model formulation, (**3**) model fitting, (**4**) model checking and, finally, (**5**) interpretation and inference.

A structured approach is taken to statistical modelling. Exploratory graphical analysis of the data is initially undertaken. For example, scatter plot matrices are used to check for correlations between variables. Transformation of data is applied where required, and then initially a full model is fitted. For example, it is proposed that the response variable is likely to be explained by some combination of a set of explanatory variables (or dummy variables) and their interactions. If the number of potential explanatory variables is small, interaction terms may be included in the initial full model. When there are many explanatory variables, choice of the full model is based upon theory, research interest and parsimony (ie. simplicity). Model selection is an interative process whereby the significance of each explanatory variable is tested using an appropriate statistic to compare nested models (see below).

After a model is selected, parameters are calculated by an appropriate method of estimation. Categorical explanatory variables (ie. multi-level variables which have discrete values) are fitted as factors, which means that a constant is estimated for each level of the factor, except the first level of the factor, which is aliased with the overall constant term. An overall significance level for a categorical variable is determined. However, each level of the variable is included when reporting the statistical model, even those that are not significant.

The test applied to assess the significance of categorical variables, as for continuous variables in a statistical model, depends on the

statistical method being used (ie. linear regression or logistic regression etc.) and this is outlined below (see 3.12.2, 3.12.3, 3.12.4 and 3.12.5).

At this stage the assumptions are tested, prior to completion of formal inference (ie significance testing and interval estimation). Checking of statistical models using diagnostic plots is necessary to:- examine the underlying assumptions of the model and data distribution, check the adequacy of the model, detect aberrant or outlying data and identify important features of the data. Three main graphical tests were applied to check statistical models. These were:- **(1)** a graph of residuals versus fitted values to test for constant variance and non-linearity (residuals should have no structure and the graph should exhibit a random pattern); **(2)** a graph of ordered residuals versus expected normal quantiles (checks for symmetry, normality and outliers), and, **(3)** a graph showing Cook Statistic versus unit index (checks for observations with high influence). Removal of outlying points and or changes to the model may be required as a result of examination of the diagnostic plots. Once a suitable model has been developed, appropriate summary graphs and tables are produced.

Model adequacy is assessed by examining:- **(1)** diagnostic plots, **(2)** standard errors associated with parameter estimates, **(3)** P values, and, **(4)** 95% confidence intervals around predicated values. The various statistical methods used in this thesis are outlined below.

3.12.2 Linear regression

Linear regression (Weisberg 1980) may be used when the expected value of the response variable changes linearly with the explanatory variables. Several assumptions must be satisfied for the use of ordinary regression including:- **(1)** the probability distribution of the response variable (y), given the covariates, should be symmetrical and continuous; **(2)** observations should be independent; and, **(3)** the variance of the data around the mean function should be constant.

The form of the model is generally:

$$y_i = \beta + \beta_1 x_{1i} + \beta_2 x_{2i} + ... + \beta_{ki} x_{ki} + \varepsilon$$

When applying ordinary regression, nested models are usually compared by calculating a variance ratio statistic, and comparing its value to an appropriate F distribution. This determines whether an explanatory variable is required in the model, ie. whether it is statistically significant.

3.12.3 Logistic regression

If the response variable has a binary outcome (eg. presence / absence data) or if binomial data is used, linear regression is inappropriate because: - (**1**) the response is not normal; (**2**) the variance function is unlikely to be constant; (**3**) the true relationship between the mean (probabilities) and the explanatory variables is likely to be non-linear. For binary data, it is generally reasonable to assume that the response can be approximated by a Binomial Distribution; this then explicitly defines the variance function (Collett 1991). The mean model takes the form:

$$\text{logit}(p) = \log(p/1\text{-}p) = b_0 + b_1 x_1 + b_2 x_2 + ... + b_n x_n$$

where p is the expected probability.

Estimation of parameters and standard errors is by iterative reweighted least squares, which means that the weights associated with each data point are proportional to the sample size. The change in residual deviance statistic, which has a χ^2 distribution, is used to compare nested models in order to select significant explanatory variables.

3.12.4 Poisson regression analysis

If counts of incidents or observations are assumed to be a function of one or more explanatory variables, ordinary least squares linear regression is unlikely to be appropriate for data analysis. This is because:- (**1**) use of a standardised linear model

may lead to negative counts, particularly when the number of counts is small, and, (**2**) the variance function will not be constant.

For count data, Poisson regression analysis (McCullagh and Neldner 1989) is often appropriate. With Poisson regression for count data, it is assumed that:- (**1**) incidents are independent and the distribution of the number of observations is Poisson, (**2**) the logarithm of the mean of the response is a function of the explanatory variables, and, (**3**) the variance of the data is equal to the mean. Estimation is by iterative reweighted least squares. Model checking is performed to determine whether the data conform to a Poisson distribution and to ensure that the assumptions of the model are satisfied. The change in deviance statistic, which has a χ^2 distribution (approximately), is used to screen potential explanatory variables for significance by comparing the change in deviance obtained by dropping (or adding) a term from the model.

3.12.5 Mixed models

When data are hierarchical (ie. when there are multiple observations on a number of subjects), it is likely that the observations within animals are more similar than observations between animals. If this dependence is ignored, serious errors can occur in inference. When data with this structure are balanced, standard analysis of variance (ANOVA) methods can be used. More often in field studies, unbalanced data occur and restricted maximum likelihood (REML) (Engel 1990) is recommended for parameter estimation. A change in deviance statistic is computed and compared to a χ^2 statistic to test the significance of the fixed effects.

3.12.6 Principal coordinates analysis (PCO)

Principal coordinates analysis (Krzanowski 1988) is a descriptive method used to reduce multidimensional data to two or three scores, such that inter-individual distances of the original multidimensional data cloud are maximised. This allows patterns existing in the original data to be seen, and associations between individuals

to be calculated. The choice of coefficient of association (similarity) is based on the question being asked of the data. For example, when examining data on the presence or absence of particular species at different sites: (**1**) the simple matching coefficient gives equal weight to conjoint presence *and* conjoint absence of species at each site, whereas, (**2**) Jaccard's coefficient gives more weight to conjoint presence of species only, ie. co-occurrence. Principal coordinate analysis requires the computation of eigen values and eigen vectors (the scores) of a suitably transformed association matrix. In this thesis PCO was used to describe:- (**1**) patterns of sites, based on similarity in the kinds of parasites present, and, (**2**) patterns of parasites, based on similarity in their distributions across sites.

3.12.7 Data summaries

Extensive analysis of data was completed for this thesis. Due to the multivariate nature of the large datasets, presentation of all results was not possible and, in general, only significant results are reported. Data from non significant results are presented only when the result was unusual or unexpected.

Table 3.1: Morphometric measurements recorded for *T. caninus*

Measure	How measured
head length	from tip of nose to external occipital protruberance
head width	measured across the head at the widest part
ear conch	measured on the inside aspect from base to tip of pinna
pes	length of the foot from the heel to the tip of the longest toe (excluding nail)
body length	from the tip of the nose to the cloaca
tail length	from the cloaca to the tip of the tail
chest girth	girth of body measured just behind the elbows
belly girth	girth of body measured immediately behind the last rib
eye size	distance between the medial and lateral canthi of the eye
testicle	length of testicle measured along the long axis, excluding the epididymis

Table 3.2: Haematological variables measured from *T. caninus*

Variable	Unit
haemoglobin	g/dl
haematocrit	(%)
red cell count (RCC)	$x10^{12}/l$
mean corpuscular volume (MCV)	fl
mean corpuscular haemoglobin concentration (MCHC)	g/dl
mean corpuscular haemoglobin (MCH)	pg
white cell count (WCC)	$x10^{9}/l$
absolute neutrophils	$x10^{9}/l$
absolute lymphocytes	$x10^{9}/l$
absolute monocytes	$x10^{9}/l$
absolute eosinophils	$x10^{9}/l$
absolute basophils	$x10^{9}/l$

Table 3.3: Serum biochemical variables measured from *T. caninus*

Variable	Unit
urea	mmmol/l
creatinine	mmol/l
bilirubin	mmol/l
gamma glutamyltransferase (GGT)	IU/l
alanine aminotransferase (ALT)	IU/l
alkaline phosphatase (ALP)	IU/l
total protein	g/l
albumin	g/l
globulins	g/l
aspartate aminotransferase (AST)	IU/l
creatine phosphokinase (CPK)	IU/l
amylase	IU/l
glucose	mmol/l
cholesterol	mmol/l
triglycerides	mmol/l
sodium	mmol/l
potassium	mmol/l
chloride	mmol/l
bicarbonate	mmol/l
calcium	mmol/l
phosphorus	mmol/l

Table 3.4: Tissues collected from necropised *T. caninus* for histopathological examination

Tissue	
liver	lung
kidney	adrenal
spleen	heart
cerebrum	cerebellum*
peripheral lymph node	mesenteric lymph node*
testicle (males)	ovary (females)
tongue	prostate*
small intestine*	colon*
stomach*	thyroid*

* not collected from all animals

Figure 3.1: Sites at which *T. caninus* was trapped

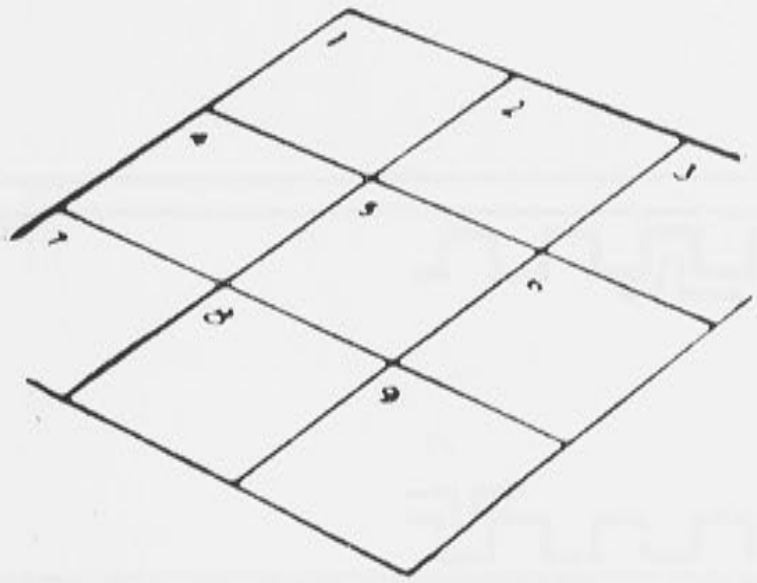
Figure 3.2: Age classes of *T. caninus* based on tooth wear of the top left first molar (adapted from Winter 1980).

Exposed dentine is black. Anterior at top, lingual at left

Wear class	Crown of M^1	Description
1		Cusps high and pointed with no apparent wear
2		Lingual cusps with points rounded but with no dentine exposed
3		Small crescents of dentine exposed on lingual cusps, but none on labial cusps
4		Crescents of dentine on lingual cusps larger, but cusps still high and rounded; dentine exposed on at least one labial cusp, but not joined to dentine crescents of lingual cusp
5		Lower limit: dentine of at least one labial cusp joined to dentine crescent of lingual cusp
6		Upper limit: dentine of lingual cusps joined, no longer appearing as crescents; dentine of both labial cusps joined to lingual cusps, but still appear as narrow strips along the cusp ridge
7		Lower limit: lingual cusps flattened, and broad band of exposed dentine between the two; dentine on labial cusps no longer a narrow strip but a broad band.
8		Upper limit: both lingual and labial cusps flattened, with large areas of exposed dentine, but still with an enamel indentation between anterior and posterior lingual cusps
9		Cusps completely obliterated and crown of tooth dished; no enamel indentation between anterior and posterior lingual cusps

Fig 3.3: Squares of a Neubauer haemacytometer.

(a) White blood cells in all nine large squares are counted.



(b) Red blood cells are counted in the four corner squares and the centre square within the large centre square of the counting chamber

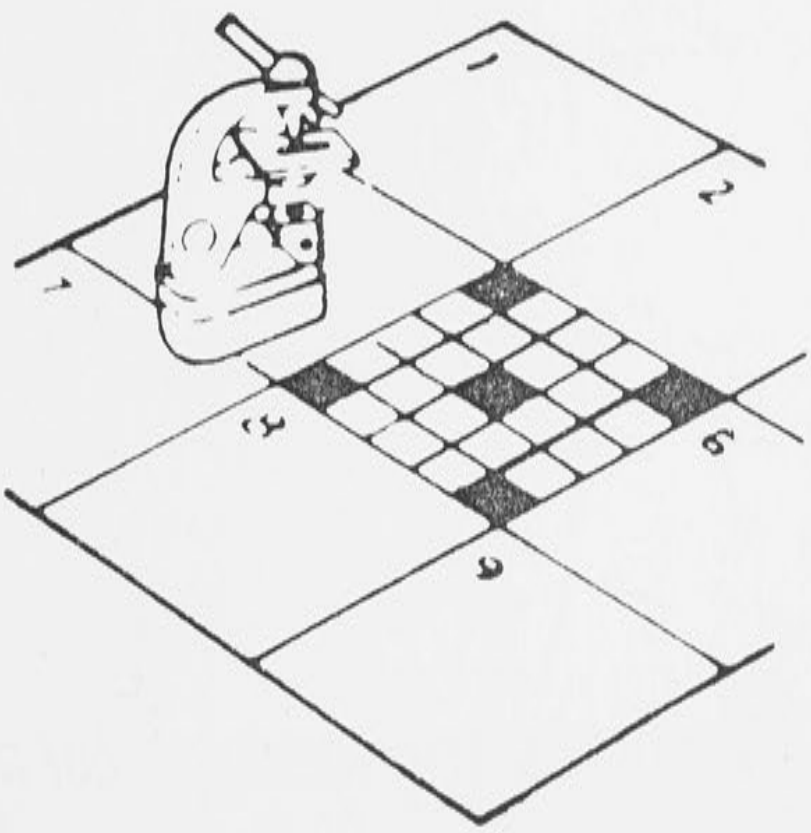


Figure 3.4: Microscope slide with thin blood smear stained with Giemsa showing Battlement method for differential white cell counts. Cells are counted in the monolayer part of the smear under X1000 magnification, following the direction of the arrows (adapted from Schalm 1965).

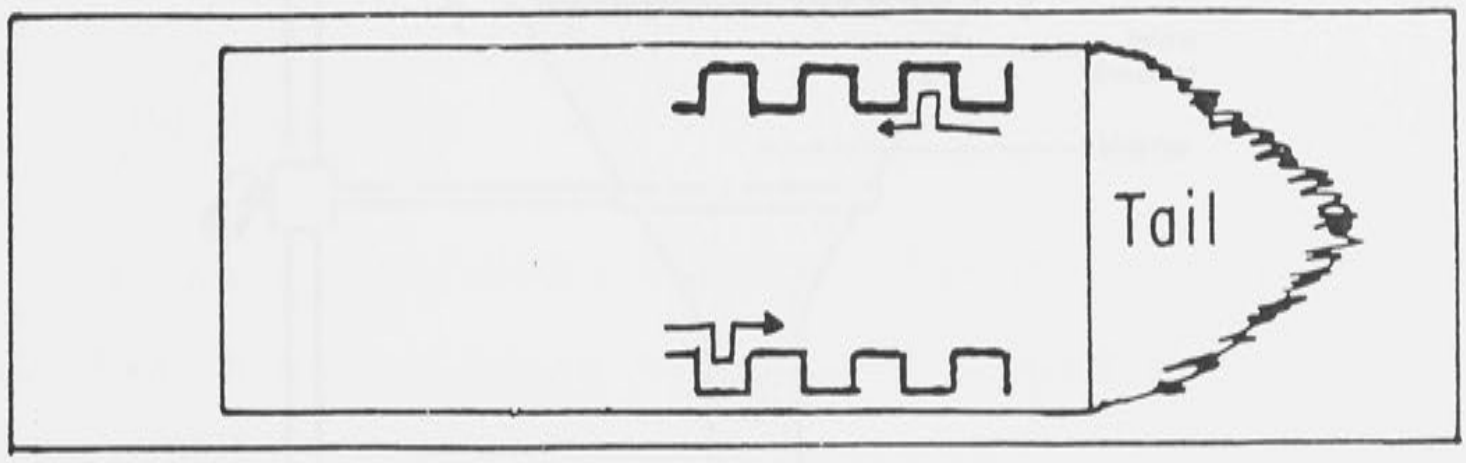
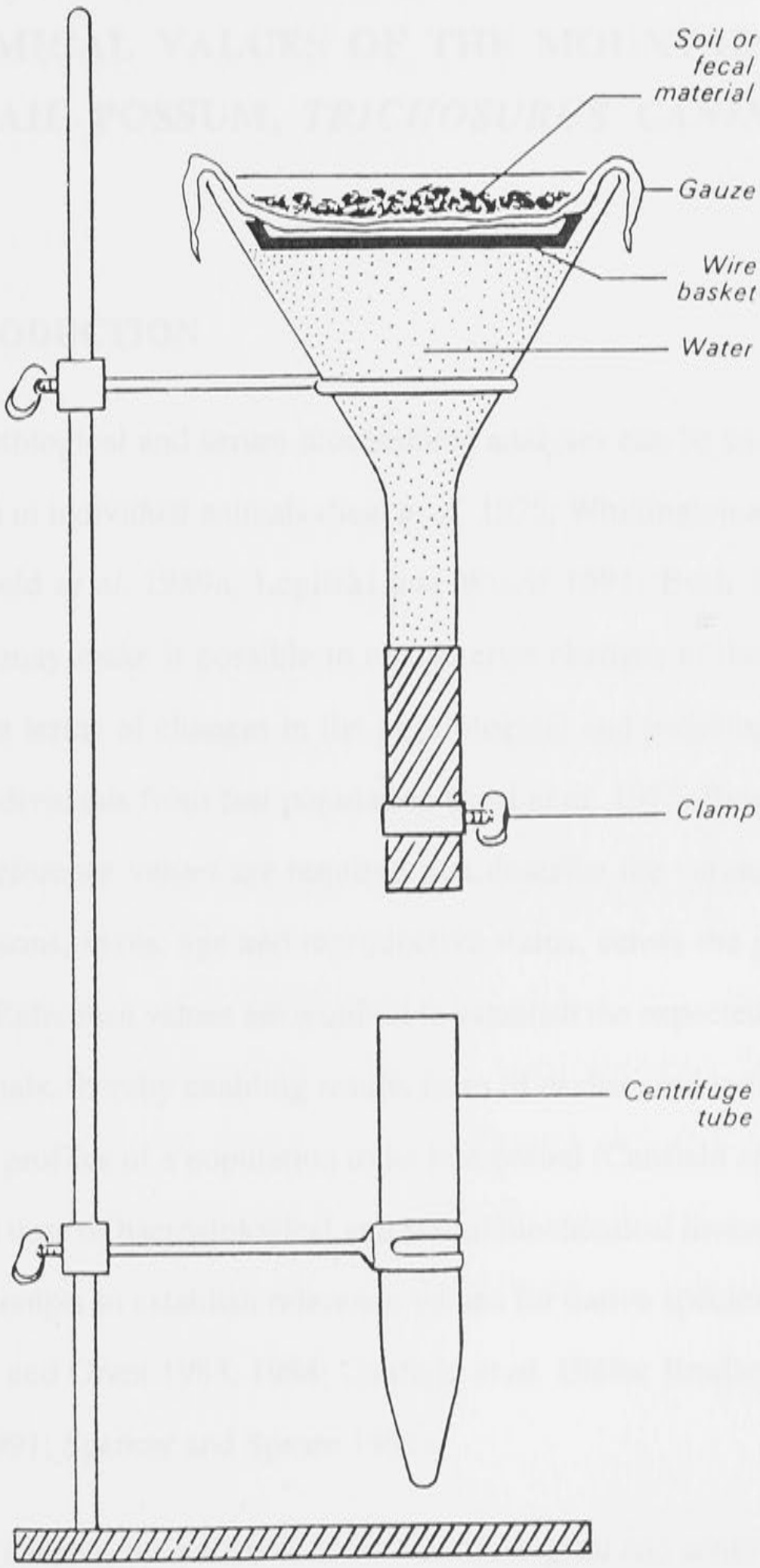


Figure 3.5: Baermann apparatus for collection of metastrongyle larvae



CHAPTER 4

VARIATION IN THE HAEMATOLOGICAL AND SERUM BIOCHEMICAL VALUES OF THE MOUNTAIN BRUSHTAIL POSSUM, *TRICHOSURUS CANINUS*

4.1 INTRODUCTION

Haematological and serum biochemical analyses can be used to detect disease and ill health in individual animals (Seal *et al.* 1975; Whittington and Grant 1983; Jain 1986; Canfield *et al.* 1989a; Lepitzki and Woolf 1991; Bush 1991). This type of information may make it possible to characterise changes in the health of a natural population in terms of changes in the physiological and pathological responses of a sample of individuals from that population (Seal *et al.* 1975; Bradley 1990). In order to do this, reference values are required that describe the variation in blood values between seasons, sexes, age and reproductive status, across the geographic range of the species. Reference values are required to establish the expected variation in normal healthy animals, thereby enabling results from ill or diseased individuals, or changes in the blood profiles of a population to be interpreted (Canfield *et al.* 1989a). Despite the potential uses of haematological and serum biochemical investigations, there have been few attempts to establish reference values for native species (Cheal *et al.* 1976; Whittington and Grant 1983, 1984; Canfield *et al.* 1989a; Bradley 1990; Haynes and Skidmore 1991; Spencer and Speare 1992).

In this chapter, the variation in the haematological and serum biochemical values of several wild populations of *T. caninus* across the geographical range of the species is examined. The first section of the study focussed on a single population of animals at Cambarville in the central highlands of Victoria. This population was trapped in each of the four seasons of the year (winter, spring, summer, autumn) and blood

samples were collected for analysis on each occasion. Significant differences in the blood values between sexes and seasons for this population are discussed and reference blood values are established for the species at this site. In addition, results of a study of haematological and serum biochemical values from *T. caninus* at six other study sites across the distribution of the species are presented, and differences between sites are discussed. Finally, blood results from two captive animals are compared with values for wild *T. caninus*.

4.2 METHODS

4.2.1 Trapping program

T. caninus was trapped at seven sites across the geographic range of the species. *T. caninus* at Cambarville were trapped during 14 day periods in June, September and December 1992 and April 1993. All animals from the six other study sites were trapped in October and November 1993. Extra data were collected from *T. caninus* at Cambarville in December 1993 for comparison of haematological and serum biochemical values between sites. The timing of collection of this additional data from Cambarville was important to minimise the confounding effect of differences between years.

Animals were sedated (see Chapter 3.3 and Appendix 2) and then weighed, measured, age-class was determined (see Chapter 3.4; Fig 3.2) and blood was collected for haematological and serum biochemical analyses (see Chapter 3.5 and 3.6). Only mature animals (≥ age-class three) were included in order to minimise potential effects of age on blood values.

4.2.2 Captive *T. caninus*

Blood samples were also collected from two captive *T. caninus* from the Healesville Sanctuary in Victoria, Australia, for comparison of haematological and serum biochemical values with those of free-ranging *T. caninus*. These were the only *T. caninus* currently held in captivity in the Australian zoological system. Animals

were sedated with tiletamine/zolazepam and blood samples were analysed as described in Chapter 3.5 and 3.6.

4.2.3 Statistical Analyses

Data collected over the four seasons from *T. caninus* at Cambarville were examined for differences between seasons, sexes, age-classes (classes 3-9, see Chapter 3.4; Fig 3.2)., excitability and the reproductive status of animals. Not all animals were captured in each of the four seasons, hence data were unbalanced and included both between and within animal variation. Restricted maximum likelihood (REML) (see Chapter 3.12.5; Engel 1990) was used to estimate random effects for between animal and within animal variability, as well as fixed effects for the factors of interest.

Reference blood values for *T. caninus* at Cambarville were determined by combining all data from all animals, including repeat measures on those individuals captured more than once, to determine the median, and 5th and 95th percentile values for each blood variable.

Results of haematological and serum biochemical analyses of *T. caninus* from seven sites across the geographic range were evaluated for significant differences between the sites, sexes and age-classes. Linear regression (Weisberg 1980) was used, because there were no repeat measures on individuals, and, in each case, the response variable (haematological or serum biochemical value) was a continuous variable that was likely to be explained by a combination of the possible explanatory variables (see Chapter 3.12.2).

Haematological and serum biochemical values were obtained for only two captive *T. caninus*. Comparisons between results for captive and wild animals were therefore descriptive only.

4.3 RESULTS

4.3.1 Cambarville

A total of 32 adult *T. caninus* (19 females and 13 males) was captured for collection of blood samples at Cambarville between June 1992 and April 1993. Most animals were trapped at least twice, giving a total of 80 captures. Haematological and serum biochemical reference values were established using data from all *T. caninus* captured (Tables 4.1 and 4.2). No significant differences between lactating and non-lactating females were found for any blood results. In addition, there was no effect of excitability rating on any blood values.

Significant differences between male and female *T. caninus* were detected for several red blood cell values (Table 4.3). Haemoglobin, RCC and haematocrit were significantly higher in males than females ($p=0.008$). Mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) were greater in females than males ($p<0.001$). MCV was lower in winter than the other seasons ($p<0.001$).

No significant differences in the total and differential white cell counts were found between the sexes or the seasons, except for the absolute eosinophil count, which was highest in autumn ($p<0.001$). Small numbers (1-5) of nucleated red blood cells were present in the smears of approximately 22% of *T. caninus* at Cambarville. In addition, Howell-Jolly bodies were present in most blood smears. Neutrophils were the predominant white blood cells in the peripheral blood of *T. caninus*.

Total protein levels were significantly higher in female *T. caninus* ($p=0.02$) (see Table 4.4). However, differences in other blood measures could not be attributed to the sex of animals (Table 4.4). Seasonal effects were detected for several biochemistry parameters (Table 4.5). Levels of urea were highest in spring ($p<0.001$) and serum protein levels were highest in summer ($p=0.002$). Total bilirubin levels were lower in summer and autumn than winter and spring ($p<0.001$), while alkaline phosphatase (ALP) was highest in summer ($p<0.001$). Lactate dehydrogenase was highest in winter ($p<0.001$). Seasonal variation was detected in serum sodium, potassium and

phosphorus levels. Serum sodium levels in *T. caninus* were highest in summer ($p<0.001$), potassium levels were highest in winter ($p<0.001$) and phosphorus levels were higher in winter and spring than the other seasons ($p=0.03$).

Variation in faecal egg counts between seasons was examined for *T. caninus* at Cambarville. Egg counts were significantly higher in spring than the other seasons ($p=0.005$) (Fig 4.1).

4.3.2 Comparisons between study sites

For the comparative aspect of this study, *T. caninus* was trapped at seven study sites: Cambarville (N=32) in central Victoria, Bellbird (N=13) in east Gippsland, Victoria, Barrington Tops (N=19) in central NSW, Whian Whian State Forest (N=7) and Byrangery Reserve (N=7) in northeastern NSW, Conondale Ranges (N=13) in southeastern Queensland, and Bulburin State Forest (N=13) in central Queensland (Table 4.6). Significant differences between the seven study sites were detected for a number of haematological and serum biochemical values (Table 4.7). For some of these variables, an equally robust, more parsimonious statistical relationship was achieved by combining the variable "site" to a new variable, "State", for animals captured at sites located in Victoria, NSW and Queensland respectively (see Table 4.8).

Haematocrit was higher in animals from Cambarville than the other study sites ($p<0.001$). Absolute eosinophil counts were higher in *T. caninus* at Cambarville than for those animals from Byrangery Reserve and Barrington Tops ($p=0.004$). Serum protein levels were significantly lower in Cambarville animals and higher in animals from Byrangery than the other sites ($p<0.001$). Serum urea levels were highest at Bellbird and lowest at Byrangery ($p<0.001$). ALT was lower in *T. caninus* at Bellbird than for animals from Cambarville and Barrington Tops ($p=0.03$).

White cell counts were higher in *T. caninus* from Queensland than the other States ($p<0.001$). Serum globulin levels were lowest in animals from Victoria ($p<0.001$), whereas serum ALP levels were significantly higher in animals from

Victoria ($p<0.001$). Cholesterol levels were lowest and triglyceride levels were highest in *T. caninus* from Queensland ($p<0.001$).

4.3.3 Captive *T. caninus*

Results of haematological and serum biochemical values for two captive *T. caninus* are given in Tables 4.9 and 4.10. There were differences in some blood values between captive animals and free-ranging *T. caninus* at Cambarville. Haemoglobin concentration, haematocrit and WCC were higher in captive animals. Lymphocytes were the predominant white blood cell type in captive *T. caninus* and absolute lymphocyte counts were much higher than those of free-ranging animals. Serum levels of AST and ALT were lower in captive animals, whereas triglyceride levels were higher than for wild *T. caninus*. Most other blood values for captive animals were within the reference ranges (5th to 95th percentiles) established for wild *T. caninus* at Cambarville.

4.4 DISCUSSION

4.4.1 Variation in haematological and serum biochemical values of *T. caninus*

Haemoglobin, RCC and haematocrit were higher in male *T. caninus* than females. Similar findings have been recorded in *T. caninus* and the closely related common brushtail possum, *T. vulpecula,* at Clouds Creek in northeastern NSW (Barnett *et al.* 1979a) and in *T. vulpecula* in good condition in Victoria (Presidente 1978; Presidente and Correa 1981). The values for MCV and MCH were higher in *T. caninus* at Cambarville captured in autumn and winter than those animals trapped during the other seasons. An increase in MCV implies an increase in the size of red blood cells, and changes in the MCV are usually accompanied by a proportionate change in MCH (Bush 1991). The cause of seasonal variation in these indices is unknown. Higher PCV values also were recorded from *T. caninus* at Cambarville than from the other study sites. However, the reason for this finding is unclear.

The values for haematocrit, RCC and haemoglobin concentration in *T. caninus* at Cambarville were similar to those reported by Presidente and Correa (1981) for *T. vulpecula* in good condition. However, the values for these variables measured from *T. caninus* at Cambarville were lower than those recorded in male animals from Clouds Creek (Barnett *et al.* 1979a). Regional variation in diet and differences in the methods of handling at capture between the study by Barnett *et al.* (1979a) study (manual restraint) and this study of *T. caninus* from seven sites (sedation with tiletamine/zolazepam) may have contributed to these differences.

Neutrophils were the predominant white blood cell in the peripheral blood of *T. caninus* at Cambarville. This differs from a number of other Australian native mammals in which a predominance of lymphocytes has been observed, e.g. *T. vulpecula* (Parsons *et al.* 1971; Presidente 1978; Presidente and Correa 1981); koala, *Phascolarctos cinereus* and southern hairy-nosed wombat, *Lasiorhinus latifrons* (Parsons *et al.* 1971); brown antechinus, *Antechinus stuartii* (Cheal *et al.* 1976); platypus, *Ornithorhynchus anatinus* (Whittington and Grant 1983); and red-tailed phascogale, *Phascogale calura* (Bradley 1990). Differential and total white cell counts were not performed in the study by Barnett *et al.* (1979a) of *T. caninus* in northeastern NSW, hence comparison with the findings for *T. caninus* at Cambarville was not possible. In captive *T. caninus*, lymphocytes were the predominant white cell type.

Neutrophil and lymphocyte values were highly variable in wild *T. caninus* at Cambarville. Some authors have highlighted the importance of lymphocyte : neutrophil ratios as indicators of disease in some animal species (eg. koala, *Phascolarctos cinereus*, Dickens 1975; Obendorf 1983). However, given the variability in these cell types in wild *T. caninus*, lymphocyte : neutrophil ratios were unlikely to be useful as indicators of disease in this species. Higher white cell counts were recorded from *T. caninus* at Byrangery Reserve in northeastern NSW than the other study sites. This may be related to the high intensity of infestation with ectoparasites, especially ticks, detected on animals at this site (see Chapter 7).

The absolute eosinophil count for *T. caninus* at Cambarville was significantly higher in autumn than the other months. Parasitism is a common cause of elevation of eosinophils in the peripheral circulation (Dawkins *et al.* 1989; Bush 1991), and may have contributed to this seasonal variation in eosinophil counts. However, faecal egg counts, which may correlate with gastrointestinal nematode burdens in some hosts (Anderson and May 1991; Gregory 1991; Stear *et al.* 1995), were highest in spring for *T. caninus* at Cambarville. The relationship between faecal egg count and intestinal parasite burden in *T. caninus* is examined in Chapter 7.4.2.

The peak of eosinophil levels in *T. caninus* in autumn could be associated with exposure to parasite larvae at this time, with peak faecal egg counts occurring in spring as a result of these infections. Buddle *et al.* (1992) found that blood eosinophil counts in lambs artificially infected with trichostrongyloid nematodes, were negatively correlated with faecal egg count, suggesting that eosinophilia was associated with the suppression of faecal egg counts. Other authors have suggested that eosinophilia may be a consequence of larvae and developing stages of trichostrongyloid nematodes embedding in the mucosa and stimulating an immune response (Kimambo *et al.* 1988).

Patterns of availability of nematode larvae for *T. caninus* at Cambarville may influence faecal egg counts and eosinophil levels. However, the life-cycles and pre-patent periods of nematodes infecting *T. caninus*, and seasonal availability of nematode larvae are unknown. Anderson (1972) found that although availability of nematode larvae to grazing sheep in western Victoria was highest in autumn and winter, faecal egg counts did not peak until spring.

Seasonal effects were detected for several serum biochemical values (see Table 4.4). Levels of urea were highest in spring and serum protein levels were highest in summer. Significantly higher serum protein levels in summer were reported by Barnett *et al.* (1979a) for *T. caninus* in northeastern NSW. Presidente and Correa (1981) did not find significant differences between the sexes in serum protein levels of *T. vulpecula*; values for urea in this species were lower than for *T. caninus* at

Cambarville. Elevated dietary protein may be a major factor influencing variation in serum urea levels (Seal *et al.* 1978; McCue and O'Farrell 1992). Seebeck *et al.* (1984) evaluated the diet of *T. caninus* at Cambarville, but these authors did not investigate the nutrient composition of dietary items (eg. protein, fat, water, etc.).

Variation in alkaline phosphatase levels may be attributed to a number of causes, including liver damage, bile duct obstruction, bone growth in young animals, starvation and diet (Bush 1991). Values for ALP generally were high in *T. caninus* at Cambarville when compared with those from other species (eg. *O. anatinus*, Whittington and Grant, 1983; *P. cinereus*, Canfield *et al.*, 1989a). These values may be normal for *T. caninus*, as animals appeared to be healthy and there was no concurrent increase in other hepatic enzymes, which may be expected if there was any associated liver pathology (Bush 1991). The ALP isoenzyme in bone may cause an elevation of serum ALP in young growing animals (Seal *et al.* 1975; Smith and Rongstad 1980). However, as only adult *T. caninus* were examined in this study, this is unlikely to be a cause of the high levels of ALP that were recorded. Serum levels of ALP were higher at Cambarville than at the other study sites. However, the cause of the higher serum levels of ALP recorded from *T. caninus* at Cambarville is not understood. Serum ALP levels of captive *T. caninus* were within the reference range established for *T. caninus* at Cambarville. Captive animals were fed a different diet to that available to free-ranging *T. caninus*, which suggests that the high ALP values observed in this species are probably not of dietary origin.

Lactate dehydrogenase (LDH) levels in *T. caninus* were highest in winter. Given that LDH is present in high concentrations in organs other than liver, (eg muscle; Bush 1991), no conclusions could be drawn as to the cause of this seasonal variation.

Serum sodium levels in *T. caninus* at Cambarville were highest in summer. Increased serum sodium levels may result from excessive water loss, decreased water intake, or increased sodium intake (Bush 1991), which may be related to seasonal dietary variation. Elevation of serum sodium levels in summer in the red kangaroo,

Macropus rufus, and the euro, *Macropus robustus*, has been attributed to variation in the dietary intake of sodium from different species of grass (Dawson and Denny 1969). Seasonal differences in sodium and potassium levels in *T. caninus* at Cambarville may be associated with fluctuating sodium intake due to seasonal variation in diet. Studies by Seebeck *et al.* (1984) and Claridge and Lindenmayer (1993, 1996), investigating the diet of *T. caninus* at this site detected significant seasonal variation in the intake of several dietary items, including fungi and the foliage of plants from the forest understorey and ground layers (see Chapter 2.6). This may have contributed to seasonal variation in serum levels of electrolytes.

Serum phosphorus levels were higher in winter and spring than the other seasons. Increased serum phosphorus may be attributable to high phosphorus levels in the diet (Bush 1991). The serum concentrations of sodium, potassium, and chloride in *T. caninus* were similar to those of *T. vulpecula* in good condition. Both calcium and phosphorus levels in *T. caninus* were lower than *T. vulpecula* in both good and poor condition (Presidente and Correa 1981).

No effect on blood values of reproductive status of female animals (lactating, non-lactating) was detected in this study of *T. caninus* at seven sites across the range of the species. Barnett *et al.* (1979b) recorded significantly lower haematocrit and plasma lipid levels in those females which lost a young and did not subsequently replace it in the same season. However, this aspect of reproductive success was not examined in *T. caninus* at Cambarville.

4.4.2 Potential influence of stress of trapping, handling and sedation on the blood values of *T. caninus*

Although it is well understood that factors such as the stress of handling and anaesthesia may significantly affect some blood values (Middleton 1976; Duncan *et al.* 1994; Bush 1991), it is not possible to complete haematological and serum biochemical studies of wild animals without handling and disturbing them (Whittington and Grant 1983). This is because wild animals are unaccustomed to

handling and human presence. Some authors have attempted to acclimatise animals under laboratory conditions for a standardised length of time prior to blood collection (Barnett 1973; Cheal *et al.* 1976, Middleton 1976). However, these results also may be difficult to interpret, as holding animals in a changed environment on a different diet probably leads to changes in the blood values. The values of some blood attributes, such as RCC, AST, CPK and glucose, may decrease over a series of trapping events as a result of animals becoming accustomed to handling (Chapple *et al.* 1991). However, *T. caninus* were trapped and handled too infrequently in our study to assess the effects of repeated short-term serial sampling on blood parameters.

Barnett *et al.* (1979a) acknowledged that the trapping and manual restraint procedures used for collection of blood from *T. caninus* at Clouds Creek, may have stressed some animals and influenced the blood results in their study. Physiological changes in blood values may have been induced by the internal release of adrenalin and glucocorticoids from the adrenal gland in response to the stress of handling (Barnett *et al.* 1979a; Bush 1991). Similarly, handling and sedation of *T. caninus* at the seven study sites in this study may have influenced some blood values. For example, handling stress may result in increases in RCC, haematocrit, haemoglobin, circulating neutrophils and lymphocytes, blood glucose, lipids and proteins (Bush 1991). Studies by Dawson and Denny (1968) of *T. vulpecula* have shown that splenic contraction due to fear and excitement gives rise to an increase in RCC, PCV and haemoglobin. In stressed or excited animals, neutrophils from the marginal pool may be temporarily redistributed to the circulating pool, causing a neutrophilia (Bush 1991). Canfield *et al.* (1989a), recorded significant elevations of neutrophil counts in koalas, *Phascolarctos cinereus*, that were struggling whilst being handled and at the time of blood collection. The neutrophil count in domestic pets may triple within six hours of a stressful event and usually returns to resting levels within 24 hours (Jacobs 1993). Physiological lymphocytosis (an increase in circulating lymphocytes as a result of fear or stress) may reach double the resting levels, and is usually resolved within 12 hours (Jacobs 1993). Given that blood samples from *T. caninus* in this study were

generally collected four to eight hours after capture, it is possible that both the neutrophil and lymphocyte counts were substantially higher than resting levels.

Other blood variables which may be affected by trap-induced stress include enzymes produced in muscle tissue, such as CPK, AST and LDH. These enzymes may increase in the peripheral circulation of animals due to muscle damage from self-trauma in the trap.

Sedation with tiletamine/zolazepam to facilitate handling of *T. caninus* may have resulted in some changes in blood values. In particular, haemoglobin concentration, haematocrit and RCC can be lower in sedated animals, due to transfer of red blood cells from the circulation to the spleen (Bush 1991). This occurs through relaxation of the splenic capsule, leading to enlargement of the spleen and an increase in splenic uptake of red blood cells (Bush 1991). Notably, the effects of sedation on blood values may occur primarily through the absence of stress (Bush 1991). Several studies on other species have not detected an effect of tiletamine/zolazepam on haematological and serum biochemical values (eg. wild dogs, *Lyacon pictus*, Van Heerden *et al.* (1991); five species of captive bears, Bush *et al.* 1980). Other anaesthetic agents have been found to significantly affect some blood values. For example, ether anaesthesia of wild platypus, *Ornithorhynchus anatinus*, caused a leucocytosis due to an increase in neutrophils and monocytes (Whittington and Grant 1984).

The following efforts were made to minimise stress prior to and during collection of blood samples from *T. caninus* in this study:- (**1**) at the time of capture animals were transferred to hessian sacks; this appeared to minimise struggling and self trauma, which were common behaviour in animals held in traps; (**2**) animals were held in a quiet place until immediately prior to sedation and blood collection; (**3**) the administration of the sedative drug was completed using minimal manual restraint with the animal sitting quietly inside the bag; (**4**) the animal was allowed to remain quietly in the bag until deep sedation had been achieved; (**5**) the animal's head was covered

by the bag at all times during handling and blood collection; and, **(6)** females and their young were not separated.

4.5 CONCLUSIONS

Significant differences between sexes and seasons in haematological and serum biochemical values were detected for *T. caninus* at Cambarville. The broad range and variability in many blood values of apparently healthy *T. caninus* may limit the use of haematological and serum biochemical investigations as a single tool for detecting disease and ill health in wild populations of this species. Serial samples may be required to identify persistent changes in blood values (Canfield *et al.* 1989b), particularly given the potential influence of stress on many blood variables. To obtain a further understanding of population and individual health in relation to blood values, a method for assessing body condition in *T. caninus* is needed. This might facilitate the identification of a number of key blood variables which reflect the health or condition of individuals and hence populations of animals (see Chapter 5).

Table 4.1: Reference red blood cell values, red cell indices, total white cell and differential cell counts for the mountain brushtail possum, *T. caninus*, (N = 80) at Cambarville, Victoria (June 1992 - April 1993).

Parameter	**Median**	**5th - 95th percentiles**
Haemoglobin (g/dl)	12.2	10.5 - 14.1
Haematocrit (%)	36.0	29.9 - 42.3
Red cell count ($x10^{12}$/l)	4.78	3.99 - 5.92
Mean corpuscular haemoglobin concentration (g/dl)	34	32 -35
Mean corpuscular volume (fl)	74.3	68.1 - 80.0
Mean corpuscular haemoglobin (pg)	25.5	23.2 - 26.7
White cell count ($x10^9$/l)	4.2	2.1 - 6.8
Neutrophilia[a]	2.1 (53%)[b]	0.5 - 4.8 (18-79%)[b]
Lymphocytes[a]	1.6 (40%)[b]	0.6 - 3.4 (18-75%)[b]
Monocytes[a]	0.1 (2%)[b]	0 - 0.5 (0-7%)[b]
Eosinophils[a]	0.1 (1%)[b]	0 - 0.5 (0-9%)[b]
Basophils[a]	0 (<0.01)	0

[a] absolute counts: units = $x10^9$/l

[b] Percent of total white blood cells

Table 4.2: Reference serum biochemical values for *T. caninus* (N = 80) at Cambarville, Victoria (June 1992 - April 1993).

Parameter	**Median**	**5th - 95th percentiles**
Urea (mmol/l)	9.5	5.8 - 15.8
Creatinine (mmol/l)	0.08	0.05 - 0.10
Total bilirubin (mmol/l)	7	2 - 18
Gamma-glutamyltransferase (IU/l)	19	13 - 39
Alanine aminotransferase (IU/l)	33	13 - 73
Alkaline phosphatase (IU/l)	1508	838 - 2977
Total protein (g/l)	62	55 - 647
Albumin (g/l)	38	34 - 42
Globulin (g/l)	24	19 - 29
Aspartate aminotransferase (IU/l)	123	79 - 240
Creatine phosphokinase (IU/l)	418	103 - 1676
Lactate dehydrogenase (IU/l)	73	15 - 627
Glucose (mmol/l)	6.8	5.7 - 9.0
Amylase (IU/l)	472	242 - 672
Cholesterol (mmol/l)	2.13	1.52 - 3.64
Triglycerides (mmol/l)	0.80	0.47 - 1.61
Sodium (mmol/l)	144	141 - 148
Potassium (mmol/l)	3.5	2.7 - 4.9
Chloride (mmol/l)	101	94 - 108
Bicarbonate (mmol/l)	30	24 - 34
Calcium (mmol/l)	2.36	2.12 - 2.53
Phosphate (mmol/l)	1.3	0.8 - 2.2

Table 4.3: Variation between sexes in the haematology of *T. caninus* at Cambarville (June 1992 - April 1993). Data were derived from 32 individuals (13 males, 19 females) and include repeat measures for animals captured more than once, giving a total of 80 measures for each blood variable.

Parameter	**Males** Mean (SE)	**Females** Mean (SE)	**p value**
Haemoglobin (g/dl)	12.7 (0.15)	11.9 (0.2)	0.008
Haematocrit (%)	37.6 (0.7)	34.9 (0.6)	0.006
Red cell count ($x10^{12}$/l)	5.1 (0.1)	4.6 (0.1)	0.007
Mean corpuscular volume (fl)	72.2 (0.6)	75.5 (0.5)	0.001
Mean corpuscular haemoglobin (pg)	24.6 (0.2)	25.9 (0.2)	0.001

Table 4.4: Variation between sexes in the serum biochemical values of *T. caninus* at Cambarville (June 1992 - April 1993). Data were derived from 32 individuals (13 males, 19 females) and include repeat measures for animals captured more than once, giving a total of 80 measures for each blood variable. Only p values for significant difference are presented.

Parameter	**Males** Mean (SE)	**Females** Mean (SE)	**p value**
urea (mmol/l)	10.3 (0.5)	9.5 (0.5)	-
creatinine (mmol/l)	0.07 (0.005)	0.07 (0.005)	-
total bilirubin (mmol/l)	8.7 (0.9)	7.9 (0.8)	-
GGT (iu/l)	19.6 (1.0)	20.6 (1.2)	-
ALT (iu/l)	39.3 (2.0)	30.6 (2.4)	-
ALP (iu/l)	1552 (95)	1658 (96)	-
total protein (g/l)	60.2 (0.7)	62.1 (0.6)	0.02
albumin (g/l)	37.2 (0.4)	37.9 (0.4)	-
globulin (g/l)	23.0 (0.6)	24.7 (0.5)	-
AST (iu/l)	154 (7)	145 (6	-
CPK (iu/l)	540 (89)	589 (63)	-
LD (iu/l)	192.9 (32.7)	213.2 (40.6)	-
glucose (mmol/l)	6.9 (0.1)	6.7 (0.1)	-
amylase (iu/l)	489 (23)	451 (20)	-
cholesterol (mmol/l)	2.3 (0.1)	2.3 (0.1)	-
triglycerides (mmol/l)	0.87 (0.10)	0.91 (0.10)	-
sodium (mmol/l)	144.7(0.4)	144.3 (0.3)	-
potassium (mmol/l)	3.7 (0.1)	3.5 (0.1)	-
chloride (mmol/l)	102 (1)	100 (1)	-
bicarbonate (mmol/l)	29.1 (0.7)	30.2 (0.7)	-
calcium (mmol/l)	2.37 (0.3)	2.34 (0.2)	-
phosphorus (mmol/l)	1.41 (0.6)	1.34 (0.6)	-

Table 4.5: Seasonal variation in haematological and serum biochemical values for *T. caninus* at Cambarville (June 1992 - April 1993)

Parameter	**Winter (N=18)** Mean (SE)	**Spring (N=21)** Mean (SE)	**Summer (N=20)** Mean (SE)	**Autumn (N=20)** Mean (SE)	**p value**
haemoglobin (g/dl)	12.2 (0.6)	12.2 (0.4)	12.1 (0.4)	12.6 (0.6)	0.08
RCC ($x10^{12}$/l)	4.9 (0.1)	4.8 (0.1)	4.7 (0.1)	4.8 (0.1)	0.001
MCV (fl)	71.0 (1.0)	74.4 (1.0)	76.7 (1.0)	73.3 (1.0)	0.001
MCH (pg)	24.5 (0.4)	25.7 (0.4)	25.7 (0.4)	25.2 (0.4)	0.001
MCHC (g/dl)	34.5 (0.4)	34.3 (0.4)	33.4 (0.4)	34.3 (0.4)	0.001
absolute eosinophils ($x10^9$/l)	0.06 (0.04)	0.05 (0.04)	0.13 (0.05)	0.21 (0.05)	0.001
Urea (mmol/l)	9.2 (0.6)	12.2 0.5)	9.3 (0.5)	8.7 (0.5)	0.001
Total bilirubin (mmol/l)	11 (1.5)	12 (1.0)	5 (0.5)	5 (0.1)	0.001
Alkaline phosphatase (IU/l)	1316 (99)	1809 (104)	2135 (107)	1120 (105)	0.001
Total protein (g/l)	61.8 (0.8)	59.0 (0.1)	62.6 (0.7)	61.2 (0.7)	0.002
Albumin (g/l)	38.7 (0.6)	35.6 (0.5)	37.6 (0.5)	38.4 (0.5)	0.001
Lactate dehydrogenase (IU/l)	481 (84)	187 (27)	96 (14)	85 (25)	0.001
Glucose (mmol/l)	7.0 (0.2)	6.5 (0.2)	6.5 (0.2)	7.1 (0.2)	0.006
Sodium (mmol/l)	147 (0.5)	143 (0.5)	144 (0.5)	145 (0.5)	0.001
Potassium (mmol/l)	4.0 (0.1)	3.5 (0.1)	3.5 (0.1)	3.4 (0.1)	0.001
Phosphate (mmol/l)	1.5 (0.1)	1.6 (0.1)	1.1 (0.1)	1.2 (0.1)	0.001

Table 4.6: Number and sex of *T. caninus* captured from each of seven study sites across the geographic range of the species

Site	Number sampled	male	female
Cambarville (Vic)	32	13	19
Bellbird (Vic)	13	8	5
Whian Whian (NSW)	7	4	3
Byrangery (NSW)	7	5	2
Barrington Tops (NSW)	19	14	5
Conondale Ranges (Qld)	13	7	6
Bulburin State Forest (Qld)	13	9	4

Table 4.7: Variation in haematological and serum biochemical values of *T. caninus* from seven study sites across the geographic range of the species

Response variable	Site C mean (SE)	BB mean (SE)	WW mean (SE)	BR mean (SE)	CD mean (SE)	BSF mean (SE)	BTP mean (SE)	p value
Haematocrit	36.7 (0.6)	31.4 (0.9)	27.5 (1.1)	30.0 (0.8)	34.0 (0.8)	31.9 (0.8)	33.3 (0.7)	0.001
log(abs. eosinophils)	-1.67 (0.09)	-1.94 (0.15)	-1.89 (0.21)	-2.30 (0.20)	-1.67 (0.14)	-1.86 (0.14)	-2.22 (0.12)	0.004
total serum protein	59.6 (0.9)	62.0 (1.5)	64.5 (2.0)	72.1 (1.8)	62.7 (1.4)	63.4 (1.4)	67.4 (1.2)	0.001
urea	10.7 (0.4)	11.7 (0.7)	8.5 (1.0)	7.9 (0.9)	10.9 (0.7)	8.8 (0.7)	8.1 (0.6)	0.001
log(ALT)	3.8 (0.05)	3.4 (0.09)	3.7 (0.12)	3.5 (0.12)	3.6 (0.09)	3.6 (0.09)	3.8 (007)	0.03

Site labels :
C = Cambarville (Vic)
BB = Bellbird (Vic)
WW = Whian Whian State Forest (NSW)
BR = Byrangery Reserve (NSW)
CD = Conondale Ranges (Qld)
BSF = Bulburin State Forest (Qld)
BTP = Barrington Tops (NSW)

Table 4.8: Variation between States in haematological and serum biochemical values of *T. caninus*

Response variable	**State** Victoria mean (SE)	NSW mean (SE)	Qld mean (SE)	**p value**
log(WCC)	1.59 (0.06)	1.66 (0.07)	2.0 (0.08)	0.001
globulin	22.7 (0.6)	31.4 (0.7)	29.7 (0.8)	0.001
log(ALP)	7.49 (0.05)	7.12 (0.06)	7.28 (0.06)	0.001
GGT	17.2 (0.7)	14.9 (0.8)	14.7 (0.9)	0.04
cholesterol	2.3 (0.07)	2.6 (0.08)	1.8 (0.09)	0.001
log(triglycerides)	-0.59 (0.06)	-0.28 (0.07)	-0.11 (0.08)	0.001

Table 4.9: Haematological values for two adult *T. caninus* held in captivity at the Healesville Sanctuary in Victoria, Australia.

Parameter	**Animal 1 (female)**	**Animal 2 (male)**	**Reference range (5th - 95th percentiles) for *T. caninus* at Cambarville**
Haemoglobin (g/dl)	15.0	18.2	10.5 - 14.1
Haematocrit (%)	43	53	29.9 - 42.3
RCC ($x10^{12}$/l)	5.08	6.66	3.99 - 5.92
MCHC (g/dl)	35	34.1	32 - 35
MCV (fl)	85	79.9	68.1 - 80.0
MCH (pg)	30	27.3	23.2 - 26.7
WCC ($x10^9$/l)	13.1	8.9	2.1 - 6.8
Neutrophils[a]	1.8 (14%)[b]	0.6 (7%)[b]	0.5 - 4.8 (18-79%)[b]
Lymphocytes[a]	11 (84%)[b]	8.0 (90%)[b]	0.6 - 3.4 (18-75%)[b]
Monocytes[a]	0.1 (1%)[b]	1.2 (2%)[b]	0 - 0.5 (0-7%)[b]
Eosinophils[a]	0.1 (1%)[b]	0.1 (1%)[b]	0 - 0.5 (0-9%)[b]
Basophils[a]	0 (<0.01)	0 (<0.01)	0

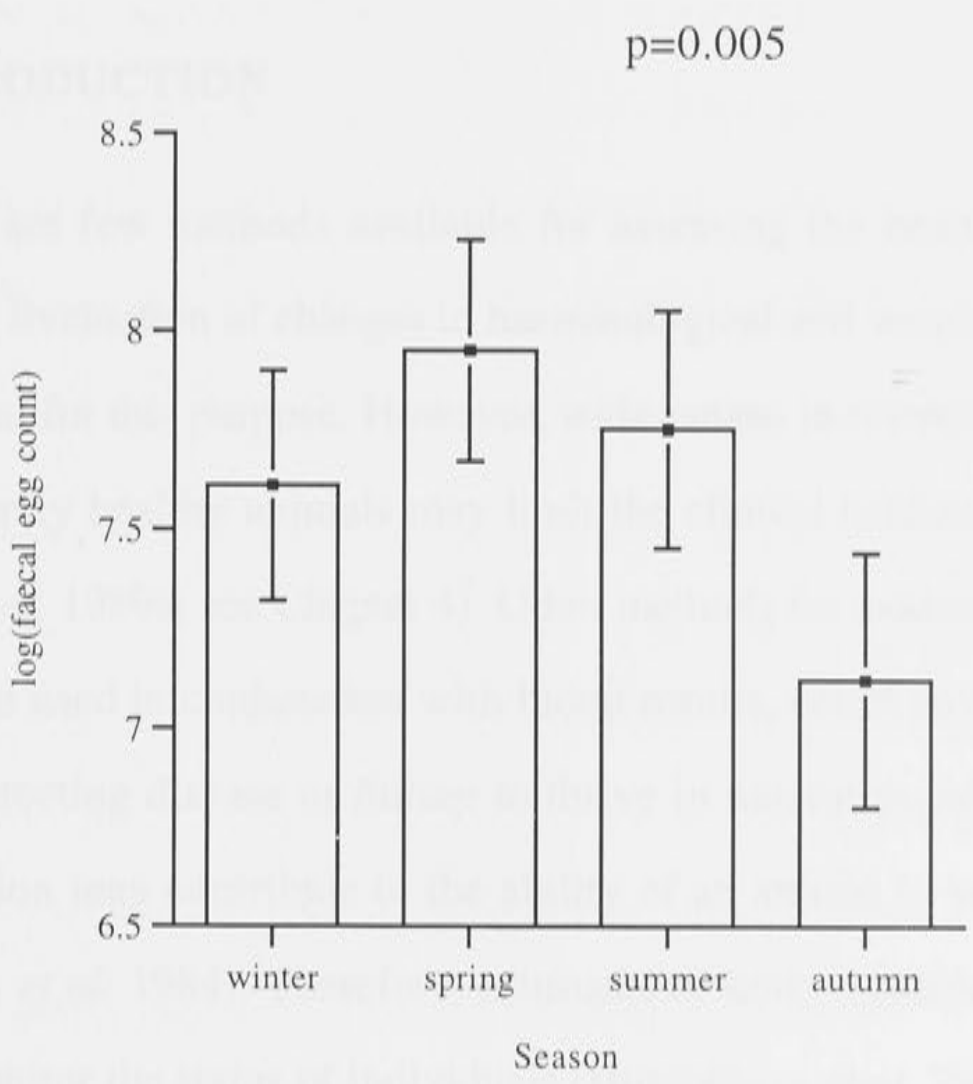
[a] absolute counts: units = $x10^9$/l

[b] Percent of total white blood cells

Table 4.10: Serum biochemical values for two *T. caninus* held in captivity at the Healesville Sanctuary in Victoria, Australia.

Parameter	**Animal 1 (female)**	**Animal 2 (male)**	**Reference range (5th - 95th percentiles) for *T. caninus* at Cambarville**
Urea (mmol/l)	5.6	4.8	5.8 - 15.8
Creatinine (mmol/l)	0.10	0.09	0.05 - 0.10
Total bilirubin (mmol/l)	3	6	2 - 18
GGT (IU/l)	17	13	13 - 39
ALT (IU/l)	<3	<3	13 - 73
ALP (IU/l)	1067	1195	838 - 2977
Total protein (g/l)	61	66	55 - 647
Albumin (g/l)	37	40	34 - 42
Globulin (g/l)	24	26	19 - 29
AST (IU/l)	34	43	79 - 240
CPK (IU/l)	413	88	103 - 1676
LDH (IU/l)	390	239	15 - 627
Glucose (mmol/l)	7.1	7.9	5.7 - 9.0
Amylase (IU/l)	723	692	242 - 672
Cholesterol (mmol/l)	2.24	2.5	1.52 - 3.64
Triglycerides (mmol/l)	3.73	1.72	0.47 - 1.61
Sodium (mmol/l)	145	145	141 - 148
Potassium (mmol/l)	4.2	3.8	2.7 - 4.9
Chloride (mmol/l)	107	107	94 - 108
Bicarbonate (mmol/l)	20	21	24 - 34
Calcium (mmol/l)	2.43	2.42	2.12 - 2.53
Phosphate (mmol/l)	1.19	0.93	0.8 - 2.2

Figure 4.1: Variation in faecal egg counts of *T. caninus* between four seasons (winter, spring, summer and autumn) at Cambarville in central Victoria



CHAPTER 5

ASSESSMENT OF BODY CONDITION IN THE MOUNTAIN BRUSHTAIL POSSUM, *TRICHOSURUS CANINUS*:

CONDITION INDICES AND OTHER ESTIMATES OF BODY CONDITION

5.1 INTRODUCTION

There are few methods available for assessing the health of animals in wild populations. Evaluation of changes in haematological and serum biochemical profiles may be useful for this purpose. However, wide ranges in reference values established from apparently healthy animals may limit the clinical usefulness of blood profiles (Canfield *et al.* 1989a; see Chapter 4). Other methods for assessing health of animals, which can be used in conjunction with blood results, could provide a more sensitive means of detecting disease or failure to thrive in natural populations. For example, body condition may contribute to the ability of an animal to survive and reproduce (Humphreys *et al.* 1984). Therefore, estimates of body condition might be useful to assess or monitor the status of individuals (Humphreys *et al.* 1984; Corn and Warren 1985; Krebs and Singleton 1993). Given this, studies of body condition may be applicable to monitoring the health status of wild populations over time (Humphreys *et al.* 1984).

In this chapter, various methods for quantifying and estimating body condition are briefly outlined and discussed. In addition, condition indices based on measures of skeletal size and body mass are derived for *T. caninus* from a study of the species at seven sites across its geographic range. Possible factors contributing to the observed

variation in condition indices are examined, and the usefulness of condition indices for assessing body condition also are discussed.

5.1.1 Condition assessment

The term 'body condition' may encompass a broad range of attributes, including health and aspects of ecological performance, such as fitness, ability to avoid or fend off predators, ability to hold a territory and ability to forage and reproduce successfully (Humphreys *et al.* 1984). Many of these attributes cannot be directly quantified by physical measures. However, some methods have been developed by various researchers to assess or quantify body condition. These methods measure or estimate fat reserves and are based on the assumption that the ability of an individual to accumulate higher fat reserves reflects a greater ability to compete, survive and reproduce.

There are numerous methods of varying usefulness for determining condition and fat reserves in animals, including:- (**1**) measurements of skin fold thickness (Durnin and Womersley 1974; Womersley and Durnin 1977; Garrow 1983); (**2**) external scoring of muscle and fat (Robinson 1960); (**3**) measurement of fat depots at post mortem (Presidente *et al.* 1982; Kie *et al.* 1983; McCown *et al.* 1991); (**4**) body composition determination by carcase desiccation (Bamford 1970; Bakker and Main 1980); (**5**) derivation of condition indices based on measures of body mass and skeletal size (eg. Bamford 1970; Garrow 1983; Krebs and Singleton 1993); (**6**) indirect estimation of fat reserves by dilution of radioactive isotopes (Vaughan and Boling 1961), and, (**7**) bioelectrical impedance analysis (Walsberg 1988). These methods will be briefly outlined and discussed.

Body fat in humans, *Homo sapiens*, is commonly estimated by measuring the thickness of skin folds. The sum of skin folds from a number of sites is then used to predict the percentage of body fat (Durnin and Womersley 1974; Womersley and Durnin 1977; Garrow 1983). A hind-leg fat index has been used to quantify condition

in terms of subcutaneous fat in Virginia opossums, *Didelphis virginiana*, in the USA (Hossler *et al.* 1994). However, this method has not been widely used or evaluated in other species.

In some animals, such as white-tailed deer, *Odocoileus virginianus* in North America, condition may be scored on the basis of an external assessment of muscle mass or back fat (Robinson 1960). Bamford (1970) observed that this method was not applicable to the common brushtail possum, *Trichosurus vulpecula*, a close relative of *T. caninus*, as the species appeared to have minimal subcutaneous fat deposits.

For some species, measures of internal fat depots that are accessible at necropsy, have been used to quantify condition; eg. kidney fat index and femur marrow fat content in white-tailed deer, *Odocoileus virginianus* (Kie *et al.* 1983; McCown *et al.* 1991); mesenteric fat, *T. vulpecula* (Bamford 1970). Presidente *et al.* (1982) established a condition score for *T. caninus* from northeastern NSW by visually assessing thoracic and peritoneal fat stores at post mortem examination.

Fat reserves may be more precisely quantified by determining the body composition of dead animals by carcase desiccation to measure water, fat, protein and mineral components of the carcase (Bamford 1970; Bakker and Main 1980; Bird *et al.* 1982; Hulbert and Grant 1983). However, frequently it is preferable to assess body condition by non-destructive means in living animals, particularly in the evaluation of endangered or threatened species.

Body fat reserves also may be estimated by using dilution of isotopic water to give an estimate of total body water space (Vaughan and Boling 1961). Studies of a diverse range of animal species have shown that there is a significant negative correlation between total body water composition and body fat composition (eg. sheep, *Ovis ovis*, Panaretto 1978; common brushtail possum, *T. vulpecula*, Bamford 1970; platypus, *Ornithorhynchus anatinus*, Hulbert and Grant 1983; eastern quoll, *Dasyurus viverrinus*, Green and Eberhard 1983; phalaropes, *Phalaropus tricolor* and

Phalaropus lobatus, Ellis and Jehl 1991; harp seals, *Phoca groenlandica*, Gales *et al.* 1994; Antarctic fur seals, *Arctocephalus gazella*, Arnould 1995; Arnould *et al.* 1996). Therefore, estimation of total body water percentage using tritiated water can give an indirect estimate of body fat. This method is used in Chapter 6 to validate the condition indices described in this chapter.

Bioelectrical impedance analysis is a more recently developed method for determining fat reserves in animals. Lean body mass is estimated from a non-invasive determination of total body electrical conductivity. An index of lipid levels is derived using differences in conductivity of lipid and lean mass (Walsberg 1988; Kuschner *et al.* 1990; Spengler *et al.* 1995). This technique has been used successfully for a number of species (eg. bears, *Ursus americanus*, *U. arctos* and *U. maritimus*, Farley and Robbins 1994; *P. groenlandica*, Gales *et al.* 1994), but was not used for *T. caninus* in this project.

Condition indices based on measures of skeletal size and body mass in live animals are widely used to quantify condition and have been derived for many species of animals (eg. cottontail rabbits, *Sylvilagus floridanus*, Bailey 1968; quokka, *Setonix brachyurus*, Bakker and Main 1980; mountain hares, *Lepus timidus*, Angerbjörn 1986; house mouse, *Mus domesticus*, Krebs and Singleton 1993; golden bandicoot, *Isoodon auratus* and northern brushtail possum, *Trichosurus vulpecula arnhemensis*, Short and Turner 1994). This is a non-invasive method whereby an index of condition is derived by examining the relationship between body mass and a measure of skeletal size, such as body length, pes (length of the foot) or head length (Garrow 1983; Krebs and Singleton 1993). In this chapter, the use of the term "condition indices", refers to indices derived in this way.

Condition indices based on measures of body size and mass have been widely used and accepted for quantifying body condition in animals. Frequently, it has been assumed, on the basis of general principles of scaling theory, that a standard weight to length ratio (body mass / length^3) is directly applicable to any species. This standard

ratio has become accepted and is often used without separate derivation for each species, and without studies to determine whether these indices are a useful measure of body condition (Krebs and Singleton 1993). Given this, a study was undertaken to derive and investigate condition indices in *T. caninus* from several study sites. Indices were derived by statistical examination of the weight/length relationship for the species.

5.2 METHODS

The aim of this study was to derive a condition index for *T. caninus* across its geographic range, and to determine which explanatory variables, if any, contributed to the observed variation in this index. A large data set of variables measured from each individual was used for these analyses, including site, sex, age-class, haematological and serum biochemical values and faecal egg count.

T. caninus was trapped at seven sites (see Chapter 3.1.2). To facilitate handling, animals were sedated by intramuscular injection of tiletamine/zolazepam (see Chapter 3.3 and Appendix 2) and a range of morphological measurements were recorded (see Table 3.2). Blood samples were collected (Chapter 3.5 and 3.6) and age-class (see Chapter 3.4), sex and reproductive status also were determined. Faeces and ectoparasites also were collected (Chapter 3.8.1 and 3.7).

Morphological data were analysed to derive a condition index. The variables 'body length' (cm) and 'body mass' (kg) were used in these analyses. Other body length measures (head length, tail length, pes) were examined, but exhibited weaker relationships with body mass. The data were log transformed to linearise the equation and then linear regression analysis (Weisberg 1980; Chapter 3.12.2) was used to determine the slope of the relationship. The condition index was expressed as the resulting ratio of body mass to length.

Variation in condition indices was then examined for effects of age, sex, site, haematological and serum biochemical values, and faecal egg count. Given that

"condition index" was a continuous response variable, and there was a large number of potential explanatory variables, linear regression was used for data analysis. Prior to analysis, data were assessed and log transformations were applied to those variables which had skewed distributions. The large number of potentially significant explanatory variables meant that subsets of data were fitted initially and variables were tested for significance using the variance ratio (F) statistic. Variables were re-tested for significance at various stages of the modelling process. Potential interaction terms among the main effects also were examined.

5.3 RESULTS

Animals were captured at seven study sites (see Table 4.6). The results of analyses of the haematological and serum biochemical values are outlined in Chapter 4 and Viggers and Lindenmayer (1996; Appendix 3). These values, as well as those for faecal egg counts, were used in the statistical analyses outlined below.

The relationship between body mass and body length was explored using linear regression analysis (Fig 5.1) and the regression equation for this relationship is given below (Equation 5.1). This result was not significantly different when the data were examined for effects of gender. This gave a relationship between body mass and body weight as shown in Equation 5.2. The standard error of the estimate of the slope of this relationship was (±0.2). A condition index for each individual was derived from this relationship (Equation 5.3).

Equation 5.1: $\log(\text{body mass}) = -8.59 + 2.1 \times \log(\text{body length})$

Equation 5.2: $\text{body mass} \propto (\text{body length})^2$

Equation 5.3: $\text{Condition Index} = \log(\text{body mass} / (\text{body length})^2)$

Haematological, serum biochemical and parasitological data, as well as site, sex and age-class data were examined using ordinary linear regression to determine which variables, if any, contributed to the observed variation in condition indices of *T. caninus*. Preliminary data analyses revealed that the seven study sites could be reduced to a new variable, "State" with three levels, encompassing those sites in Victoria, New South Wales and Queensland respectively. On the basis of diagnostic plots, one observation with high leverage was removed and subsequent diagnostic plots indicated that the final model was robust. Table 5.1 shows the regression coefficients and standard errors for significant explanatory variables in the final statistical model.

Four variables were found to be significant. These were:- (**1**) State (Victoria, NSW, Queensland); *T. caninus* in Victoria had significantly higher condition indices than those in NSW and Queensland ($p<0.001$; Fig 5.2a); (**2**) the log of the absolute neutrophil count; condition index was positively correlated with log(absolute neutrophils) ($p<0.001$; Fig 5.2b); (**3**) the log of the faecal egg count; a negative relationship was found between condition index and log(faecal egg count) ($p<0.001$; Fig 5.2c); and, (**4**) total serum protein; there was a positive relationship between condition index and serum protein levels ($p=0.005$; Fig 5.2d).

5.4 DISCUSSION

Condition indices are often used to quantify body condition in individuals and wild populations (Bailey 1968; Angerbjörn 1986; Lehmann 1992; Short and Turner 1994). In this study, we derived condition indices for *T. caninus* employing techniques used by numerous other researchers (see Bakker and Main 1980; Krebs and Singleton 1993). Several previous studies have reported a relationship between body mass and a particular measure of skeletal size, of body mass divided by length cubed, ie. body mass / length^3 (eg. *T. vulpecula*, Bamford 1970; *Setonix brachyurus*, Bakker and Main 1980). Numerous subsequent studies have assumed that this relationship applied to other species without independent derivation to verify the index (eg. Lehmann 1992; Short and Turner 1994). In this study of *T. caninus*, body mass

was related to the square of the body length. This finding differs considerably from the standard accepted index and emphasises that, if condition indices are to be used as a measure of condition, they must be independently derived for each species.

Data analysis using linear regression identified four significant variables which contributed to the variation in condition indices of *T. caninus*. These were: State, log(absolute neutrophils), log(faecal egg count) and total serum protein. Subjective visual appraisal of coat condition and clinical veterinary assessment of animals from the different States gave the impression that animals in Victoria were in better condition than those in NSW and Queensland. Condition indices also were significantly higher for *T. caninus* in Victoria. Habitat differences and variation in food resources across the distribution of the species may have contributed to the observed variation in condition indices between States.

There was a significant positive relationship between condition indices and log(absolute neutrophils). However, the reason for this relationship is difficult to determine. Several factors may contribute to the number of neutrophils circulating in the peripheral blood, including inflammation, stress, and systemic disease (Bush 1991). Although all animals were subjected to similar handling procedures, the stress of handling may have contributed to variation in neutrophil counts by causing a neutrophilia (see Chapter 4). However, it is unclear why those animals with higher condition indices had higher neutrophil counts.

There was a significant negative relationship between faecal egg count and condition indices of *T. caninus*. Those animals shedding more eggs in their faeces may be carrying higher burdens of parasites, which may be reflected in their poorer condition. An examination of faecal egg counts and actual parasite burdens from a post mortem sample of *T. caninus* (N=26) (see Chapter 7), did not reveal a significant relationship. However, the sample size in that analysis was small, and studies of other species have demonstrated a significant relationship between faecal egg count and

intestinal parasite burden (Stear *et al.* 1995). McCown *et al.* (1991) reported a significant negative relationship between the number of parasites in the abomasum (stomach) of white-tailed deer, *O. virginianus*, and physical condition, estimated by subjective assessment of internal fat depots at post mortem. Animals in good physical condition carried low burdens of abomasal parasites, whilst those in poorer condition had higher counts (McCown *et al.* 1991). Further studies on larger numbers of *T. caninus* would be required to establish whether faecal egg counts reflect intestinal parasite burdens. However, given the wide range of variables which contribute to survival and fecundity of helminths in the host (see Chapters 7 and 8), substantial variability in faecal egg counts would be expected.

There was a significant positive relationship between condition indices and total serum protein levels of *T. caninus*. Higher serum protein levels may indicate higher levels of protein in the diet. Franzmann and LeResche (1978) found that total serum protein was higher in Alaskan moose, *Alces alces gigas*, that were in better condition (graded by external assessment). These authors suggested that this parameter may be useful to monitor moose populations, because of the tendency of total serum protein to reflect the nutritive status of the animal. In addition, Messier *et al.* (1987) reported a significant positive relationship between total serum protein and the fat content in the carcase of caribou, *Rangifer tarandus*. Findings from these studies indicate that the relationship between condition indices and total serum protein for *T. caninus* may reflect higher fat reserves or at least a higher nutritional level in those animals with higher protein levels.

The interpretation and usefulness of condition indices relies on assumptions that these indices reflect:- (**1**) the actual body condition or fat reserves in an animal, and, (**2**) the relative fitness of individuals within a population (Brochu *et al.* 1988; Krebs and Singleton 1993). Many authors assume that this is a valid method for quantifying condition without confirmation by other methods (Bailey 1960; Angerbjörn 1986; Short and Turner 1994; Chastel *et al.* 1995). However, inherent

genetic variation between individual animals may result in variation in body shape and size which is not due to fat depots and this may influence values for condition indices. Variation in body protein and water content can also contribute significantly to body mass changes (Farley and Robbins 1994; Arnould 1995). In addition, factors such as variability in gut fill may influence measures of body mass in animals, particularly large herbivores, and this may confound estimates of condition indices (Krebs and Singleton 1993). Given this, other tests should be used to determine whether condition indices are useful measures of condition, such as methods to ascertain whether indices are correlated with fat reserves.

5.5 CONCLUSIONS

Condition indices should be independently derived from body measurements and body mass for each species (Krebs and Singleton 1993; Blakely and Kirkwood 1995). In addition, the results of this study suggest that an independent measure of body composition or fat, should be used to test the validity of the derived index as a measure of body condition in terms of body fat (Bakker and Main 1980; Krebs and Singleton 1993). In Chapter 6, condition indices derived for *T. caninus* are tested to determine whether they are significantly correlated with body fat.

Table 5.1: Regression coefficients and standard errors for significant explanatory variables in a statistical model of the variation in Condition Index for *T. caninus* from seven study sites, using linear regression (also see Figure 5.2).

Variable	Regression coefficient	SE
Constant	1.270	0.130
State Vic	0*	-
State NSW	-0.075	0.027
State Qld	-0.103	0.024
log(neutrophils)	0.053	0.018
log(faecal egg count)	-0.033	0.0092
total protein	0.0049	0.0018

* The estimate of the regression coefficient for the first level of the categorical variable "State" was set to zero because of constraints due to degrees of freedom.

Figure 5.1: Fitted values from regression analysis of the relationship between log of body mass and log of total length of *T. caninus* from seven study sites in eastern Australia; $p<0.001$, $r^2=0.57$

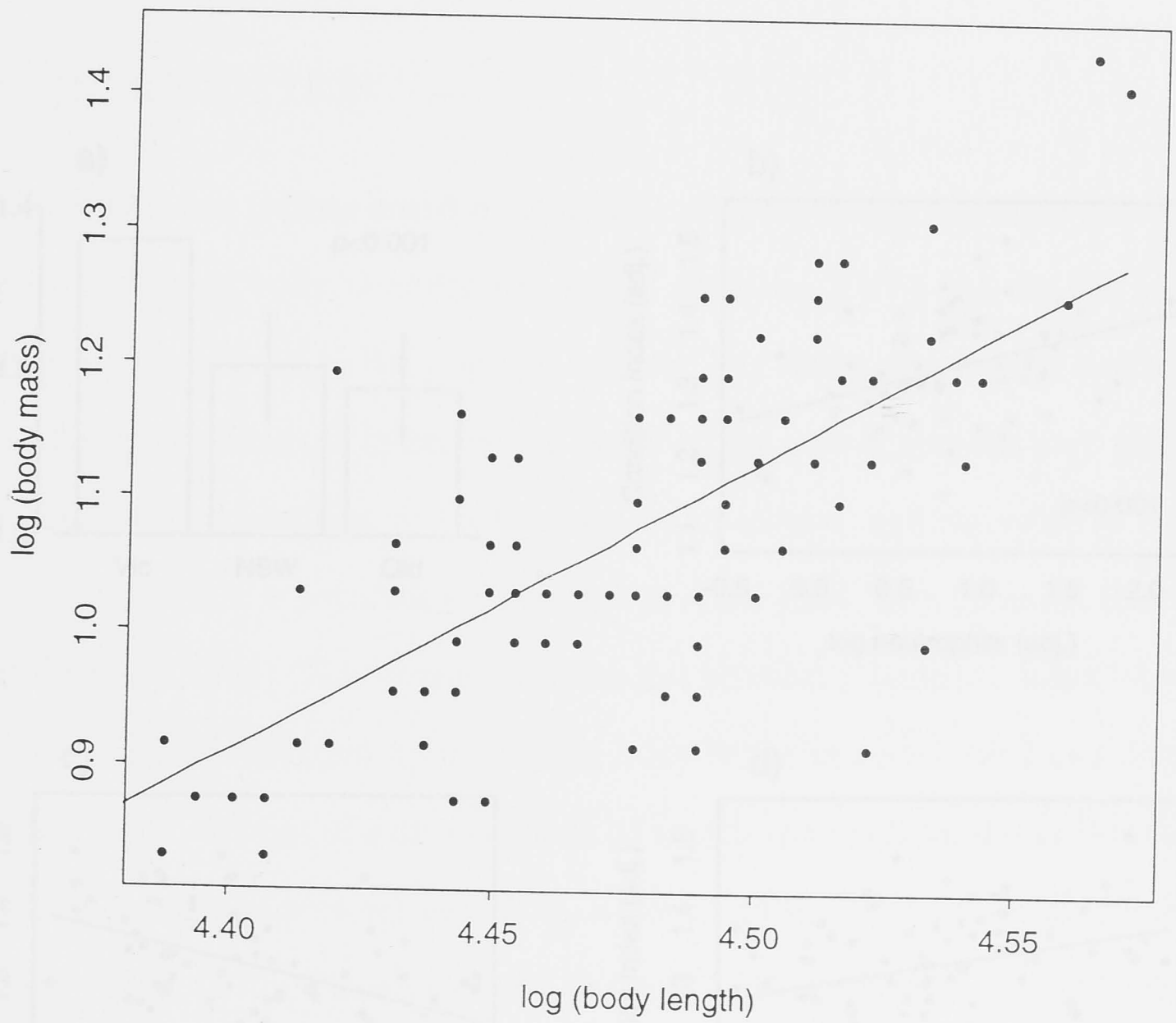
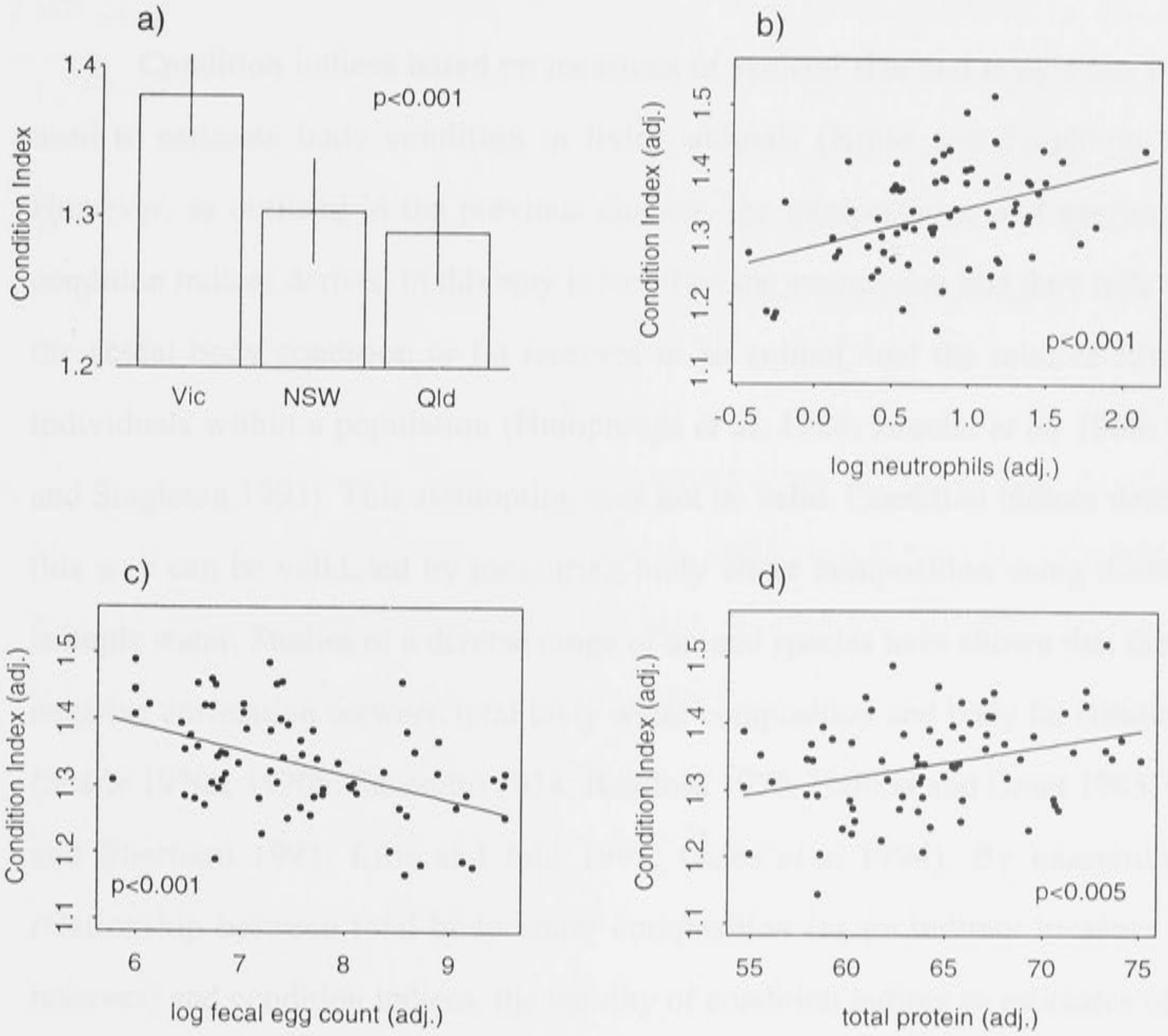


Figure 5.2: Added variable plots for significant explanatory variables in a statistical model examining variation in condition indices of *T. caninus* in eastern Australia (values for each variable are adjusted for the presence of the other explanatory variables); **a)** effect of location (State) (mean ±SE), **b)** relationship between condition index and the log of the absolute neutrophil count, **c)** relationship between condition index and log of faecal egg count, **d)** relationship between condition index and total serum protein levels (see Table 5.1).



CHAPTER 6

VALIDATION OF CONDITION INDICES FOR THE MOUNTAIN BRUSHTAIL POSSUM, *TRICHOSURUS CANINUS*, USING DILUTION OF ISOTOPIC WATER TO ESTIMATE BODY WATER COMPOSITION

6.1 INTRODUCTION

Condition indices based on measures of skeletal size and body mass may be used to estimate body condition in living animals (Krebs and Singleton 1993). However, as outlined in the previous chapter, the interpretation and usefulness of condition indices derived in this way is based on the assumption that they reflect both the actual body condition or fat reserves in an animal, and the relative fitness of individuals within a population (Humphreys *et al.* 1984; Brochu *et al.* 1988; Krebs and Singleton 1993). This assumption may not be valid. Condition indices derived in this way can be validated by measuring body water composition using dilution of isotopic water. Studies of a diverse range of animal species have shown that there is a negative correlation between total body water composition and body fat composition (Searle 1970a, 1970b; Panaretto 1978; Bamford 1970; Hulbert and Grant 1983; Green and Eberhard 1983; Ellis and Jehl 1991; Gales *et al* 1994). By examining the relationship between total body water composition (as an indirect measure of fat reserves) and condition indices, the validity of condition indices as estimates of body fat reserves can be assessed (Bakker and Main 1980).

In Chapter 5, condition indices were derived for *T. caninus* from the relationship between body mass and body length measurements for individuals from seven sites across the distribution of the species. In this chapter, the condition indices derived for a single population of *T. caninus* at Cambarville in the central highlands of

Victoria, were tested for correlation with body fat, by using isotopic dilution of tritiated water to give an estimate of total body water space. Body water composition of *T. caninus* measured in two seasons (winter and spring) was examined for effects of season, sex, reproductive status, blood values and faecal egg count.

6.2 METHODS

6.2.1 Capture, restraint and sample collection

T. caninus was captured during a two week period in each of two seasons, winter (June) 1995 and spring (November) 1995 at Cambarville in the central highlands of Victoria. After capture, each animal was manually restrained in a bag and 37MBq of tritiated water was administered by intramuscular injection (total volume per animal = 250µl). Animals were then held in a cool, quiet place with no access to food or water for three hours to allow equilibration of the tritiated water in the body water pool.

After three hours, animals were sedated by intramuscular injection of tiletamine/zolazepam (see Chapter 3.3 and Appendix 2). A total of 1ml of whole blood was collected from each animal that had been injected with tritiated water and was transferred to a cryotube and frozen in liquid nitrogen for later determination of tritiated water content. In addition, in spring, a range of skin folds was measured from each animal using Vernier calipers (see Table 6.1). For each skin fold, measurements were made on each side of the body and a score was determined by averaging the two measurements.

6.2.2 Tritiated water analyses

In the laboratory, water was extracted from each blood sample by vacuum sublimation (Vaughan and Boling 1961). Glass tubes with a Thunberg closure were used for this procedure (Fig 6.1). The necks of the sample and collection tubes were greased using high vacuum grease in order to achieve an adequate seal. Each sample

consisted of a cryotube containing approximately 1.0ml of whole blood, which was placed in the sample tube (see Fig 6.1). The sample tube was then placed into a flask of liquid nitrogen for about 20 seconds to freeze the sample. A vacuum seal was then established using a water-generated vacuum pump as follows. The side arm of the Thunberg closure was attached to a line leading directly to the vacuum pump. The Thunberg closure was then rotated to the open position to evacuate air from the tube unit. After allowing 30-60 seconds for a partial vacuum to be established, the arm was rotated to the closed position, and the vacuum pump was detached. The collection tube end of the sublimation apparatus was then partly immersed in liquid nitrogen as shown in Fig 6.1. The lowered pressure (resulting from the vacuum) and the lowered temperature inside the tube system allowed the blood sample to be sublimated, thus extracting water from the blood sample. Water vapour escaping from the sample, was frozen in the collection tube. The sublimation unit was allowed to stand overnight, with the end of the collection tube immersed in liquid nitrogen to complete the extraction of water. Thawing was completed the next morning at room temperature. The sublimated tritiated water was then collected and transferred to an eppendorf tube. For each sample, a 100µl aliquot of the collected sample of tritiated water was added to a scintillation vial containing 3 ml of scintillation cocktail (PCS, Amersham). Duplicate samples were prepared for counting for each animal.

A standard for measurement of tritium radioactivity was made by adding 250µl of tritiated water (total 37 MBq) to one litre of distilled water and mixing well to avoid error due to fractionation. Standards were prepared for counting using the methods described above for the tritiated water samples (ie. 100µl was added to scintillation cocktail). Blank vials, containing distilled water and scintillation cocktail, were also prepared to give a measure of the background radioactivity of the scintillation counter. Tritium radioactivity in each of the sample, standard and blank vials was counted for 20 minutes, using a liquid scintillation counter (Beckman LS 2800), giving an error estimate of ±1%. All samples were made in duplicate and counted twice.

Tritiated water space (HTO space) was determined by comparing the specific radioactivities of diluted standards with those of the tritiated water from blood samples collected after isotope equilibration in *T. caninus*. Total body water (TBW%) was estimated by calculating HTO space (litres) as a percentage of the body mass of the individual.

$$\text{HTO space (litres)} = \frac{\text{mean of standard counts - mean of blank counts}}{\text{mean of sample counts - mean of blank counts}}$$

6.2.3 Statistical analyses

Morphological data from both seasons for all individuals were combined, and condition indices were derived to examine the relationship between body mass and body length. Some, but not all, *T. caninus* were captured in both trapping periods, so the data were unbalanced, and mixed models were used to take account of both within and between animal variation (Engel 1990) (see Chapter 3.12.5).

To determine whether there was a significant relationship between condition indices and body water composition, the relationship between the condition indices of *T. caninus* captured in two seasons and TBW% was investigated using mixed models.

Variation in TBW% in *T. caninus* between the two seasons was then examined. These data also were unbalanced and mixed models (REML) were used to determine the significance of sex, season, haematological and serum biochemical values, and faecal egg count on TBW%. Initial analyses indicated that the between animal component of the variance was negligible (negative variance component), therefore linear regression (Weisberg 1980; see Chapter 3.12.2) was used to explore relationships in the data.

Measures of skin fold thickness were examined for correlation with TBW%; ie. to determine whether any combination of skin folds could be used to quantify body fat. Given the multivariate nature of the skin fold data, principal coordinate analysis (Krzanowski 1988; Chapter 3.12.6) was used to facilitate visualisation of associations between skin fold measurements. As TBW% and skin fold measurements were continuous variables, and because there were no repeat measures, linear regression was used to examine the relationship between TBW% and each sum of skin folds.

6.3 RESULTS

Values for tritiated water space were determined for *T. caninus* from Cambarville captured during two seasons:- winter (N=26) and spring (N=26). Results of these analyses indicated that the values for TBW% in *T. caninus* were 68.4 ± 0.5 (mean ± SE), with a range of 63.4 - 72.3 in winter; and 71.7 ± 0.5 (mean ± SE), with a range of 64.4 - 78.5 in spring.

Condition indices also were derived using the methods outlined in Chapter 5. The equation for the relationship between body mass and total length is given in Equation 6.1. The standard error of the estimate of the slope of the relationship was (±0.3).

Equation 6.1 : log(body mass) = 1.1 + 1.7 x log(body length)

The result for the relationship between body mass and body length in *T. caninus* captured during winter and spring at Cambarville was not significantly different from that of *T. caninus* from seven study sites across the geographic range of the species (see Chapter 5, Equation 5.1).

The relationship between condition index and TBW% was then examined. There was no significant relationship between these two variables (p=0.08; Fig 6.2).

Mixed models were used to examine the variation in TBW% for *T. caninus* over the two seasons. The significant explanatory variables in the final model were: season, log of absolute neutrophils and urea (Table 6.2). *T. caninus* had significantly higher values for TBW% in spring than in winter ($p<0.001$), indicating that fat reserves were generally lower in spring (Fig 6.3a). Animals with higher TBW% had lower absolute neutrophil counts ($p=0.007$) (Fig 6.3b) and higher urea values ($p=0.01$) (Fig 6.3c).

Skin fold variables were examined using principal coordinates analysis to detect patterns of similarity and association between individual variables. No distinct associations of skin fold measures were apparent. A number of sums of skin folds (tail + groin; leg + tail; leg + groin) were examined using linear regression for correlation with TBW%. However, there were no significant relationships between any combinations of skin fold scores and TBW%.

6.4 DISCUSSION

6.4.1 Body water composition

Values for TBW% in *T. caninus* at Cambarville in winter (68.4 ± 0.5), were similar to those reported by Kennedy and Heinsohn (1974) for non-lactating *T. vulpecula* (68.6 ± 1.8). These authors found that TBW% in *T. vulpecula* was considerably higher (ie. body fat composition was lower) for lactating animals (78.1 ± 3.5). Although all female *T. caninus* at Cambarville were lactating, there was no significant difference between the sexes in TBW%.

Cowan (pers. comm.) demonstrated that for the common brushtail possum, *T. vulpecula*, although tritiated water over-estimated body water by approximately 5%, body water composition, measured by both carcase desiccation and dilution of isotopic water, was negatively correlated with body fat composition (Fig 6.4). Given that *T. caninus* is morphologically similar and closely related to *T. vulpecula*, the results of Cowan's unpublished work suggest that body water estimation by dilution of isotopic water is probably a valid method for estimating body fat reserves for this species.

Similar research to validate this method in *T. caninus* could not be completed due to difficulties in obtaining permits to euthanase animals of this species, which are protected in Australia.

Given the inverse relationship between body fat and TBW%, it would be expected that if condition indices reflected body fat in *T. caninus*, there should be a significant negative relationship between TBW% and condition indices (see Bakker and Main 1980). However, evidence for such a relationship between these two variables for the population at Cambarville was scant, displayed much scatter and was not significant (p=0.08; Fig 6.2). There is little likelihood of a Type II error in this interpretation of the data given the large spread in the data points around the mean relationship. Hence for this population, condition indices do not appear to reflect body fat. If body condition is defined as the level of body fat reserves, condition indices do not appear to be useful as an estimate of condition for *T. caninus* at this site. There were also no significant relationships between any combinations of skin fold scores and TBW% for *T. caninus* at Cambarville. This indicates that measures of skin fold thickness are unlikely to be a useful measure of body fat reserves in this species. Given this, TBW% appears to be a better and more direct measure of body condition in terms of fat reserves in *T. caninus* than either condition indices or skin folds.

6.4.2 Regression analyses of TBW% for *T. caninus* at Cambarville

Significant explanatory variables in a statistical model to examine variation in TBW% using linear regression were:- season, log of neutrophils and urea (Table 6.2 and Figs 6.3a,b,c). Higher TBW% values were recorded for *T. caninus* in spring, indicating that animals had lower fat reserves at this time. The reasons for this are not clear, but several factors may have contributed to this result. Although spring is probably a period of plant growth and improved food availability, higher densities of animals sharing the study site and food resources at this time may have contributed to the lower estimates of fat depots that were observed. Alternatively, lower fat depots in spring may have resulted from depletion of fat stores over the cold, wet winter that is

typical of this region (Seebeck *et al.* 1984). Animals may be carrying greater fat stores in autumn as a legacy of preparing for winter by consuming large quantities of *Acacia* spp. seeds (Lindenmayer, pers.comm.).

No significant differences in TBW% were found in winter or spring between male and female *T. caninus*. This is surprising, as adult females were carrying advanced pouch young or dependent back young in spring. Spring is the period of peak lactation (see Green *et al.* 1988) and would be expected to have contributed to lower fat reserves in lactating animals at this time. Studies of Bennett's wallaby, *Macropus rufogriseus*, (Loudon 1987) have shown that maternal energy requirements increase dramatically when pouch young are in the advanced stages of development up to the time of emergence. Hulbert and Gordon (1972) found that lactating short-nosed bandicoots, *Isoodon macrourus*, had high values for TBW%, which reflected low body fat reserves.

The underlying physiological reason for the significant negative relationship between TBW% and the log of absolute neutrophils is unclear. A positive relationship was found between condition index of *T. caninus* and the log of absolute neutrophils (see Chapter 5); which suggests that this variable may be important. Larger or fatter animals may have higher neutrophil counts, or may be more readily able to mobilise neutrophils from the marginated pool during the stress of handling and sedation.

There was a significant positive relationship between TBW% and serum urea levels in *T. caninus* at Cambarville. For some species, serum urea levels may reflect protein intake when energy intake is above maintenance requirements (Kirkpatrick *et al.* 1975; Seal *et al.* 1978). However, in other species, a high energy diet may suppress serum urea levels, whilst restricted energy intake may cause it to increase (Kirkpatrick *et al.* 1975; Warren *et al.* 1982; Corn and Warren 1985). In addition, animals on low energy diets may catabolise body protein as an energy source, resulting in elevated serum urea levels (Kie *et al.* 1983; Corn and Warren 1985). Therefore, an understanding of the relationship between serum urea levels and TBW%

would require a detailed study of the metabolism of *T. caninus* and knowledge of the energy and protein levels in the diet.

6.4.3 Usefulness of condition indices

In some species, estimation of tritiated water space has been used to confirm the validity of other methods of quantification of fat reserves. For example, condition scoring on the basis of a tail fat index in the platypus, *Ornithorhynchus anatinus*, was established by confirming a negative correlation between TBW% and the relative volume of the tail (Grant and Carrick 1978). Although condition indices did not correlate significantly with fat depots in *T. caninus*, their usefulness should not be totally discounted. Condition indices represent a method by which the body mass of an animal is related to its length. Variation in body structure can encompass a wide range of variation other than fat reserves, including morphometric variation between individuals, skeletal structure, muscle wastage and protein loss, individual variation in organ weights and variation in stomach contents. Given this, it is not surprising that condition indices do not necessarily reflect body fat reserves. In addition, there are many sources of variation between individuals which are not measurable. These include:- inherent variation in individuals between fat depots, metabolism and other factors, such as social status. However, despite the sources of variability between individuals, repeat scoring of condition indices may be useful for monitoring specific individuals within a population over time.

6.4.4 Limitations of HTO analysis.

Variation in the intestinal fill of different individuals may contribute to error in the estimation of TBW%, as some of the water equilibrates in the intestinal contents and is therefore lost to measurement (Panaretto 1978). Prolonged fasting prior to measurement of TBW% (18 hours) may help to reduce this error (Panaretto 1978) but may be impractical when working with wild animals in the field. *T. caninus* captured in this study had no access to food for between 12-16 hours prior to blood sampling.

This represents the possible time elapsed from the time of capture, through clearing of traps, equilibration of tritiated water and eventual blood sampling.However the effect of variation in gut fill on the total body water estimates for these animals is not possible to determine.

Other factors such as variability in the moisture content of the food, seasonal variation in diet and amount of food eaten also may contribute to variation in estimates of tritiated water space (Ellis *et al.* 1995). Despite these limitations, Cowan's unpublished data for *T. vulpecula* show that TBW% is correlated with body fat in this species, and confirm that it is a useful method for estimating body water composition.

6.5 CONCLUSIONS

Condition indices were derived for *T. caninus* at Cambarville from a regression of body mass and body length. However, testing of these indices using isotopic dilution of tritiated water indicated that there was no correlation with body fat depots. The usefulness of both of these methods for estimating condition relies on the assumption that fitness or condition of animals is related to amount of body fat. This assumption may not be correct. More specific methods are required to quantify or assess both health and condition. It is likely that a combination of factors will be needed to determine an overall assessment of fitness or condition of an animal.

Table 6.1: Sites of skin folds measured from *T. caninus* at Cambarville

Fold	Site
leg fat index	skin fold of hind leg at back of stifle[a]
shoulder	skin fold over middle of spine of scapula
humerus	skin fold over middle of humerus, lateral side
elbow	skin fold at back of armpit
groin	skin fold in inguinal region of groin
tibia	skin fold from cranial aspect of mid tibial region
tail	skin fold at lateral aspect of base of tail

[a] The thickness of skin and subcutaneous fat on the posterior portion of each animals thighs was measured at two points with calipers and these four measurements were averaged to provide a leg fat index (from Hossler *et al.* 1994).

Table 6.2: Regression coefficients and standard errors for significant explanatory variables in a statistical model of the variation in percentage total body water (TBW%) in *T. caninus* measured in winter and spring at Cambarville in central Victoria (see also Fig 6.3).

Variable	Regression coefficient	SE
Constant	66.48	2.01
season	3.288	0.737
absolute neutrophils	-1.021	0.365
urea	0.454	0.174

Figure 6.1: Rapid vacuum sublimation unit. **A** Sublimation tube (sample tube). **B** Receiver for the condensed water vapour (collection tube). During sublimation the tip of B is just immersed in liquid nitrogen, the sample is placed in A, and the side arm valve (Thunberg closure) is closed. (Adapted from Vaughan and Boling 1961).

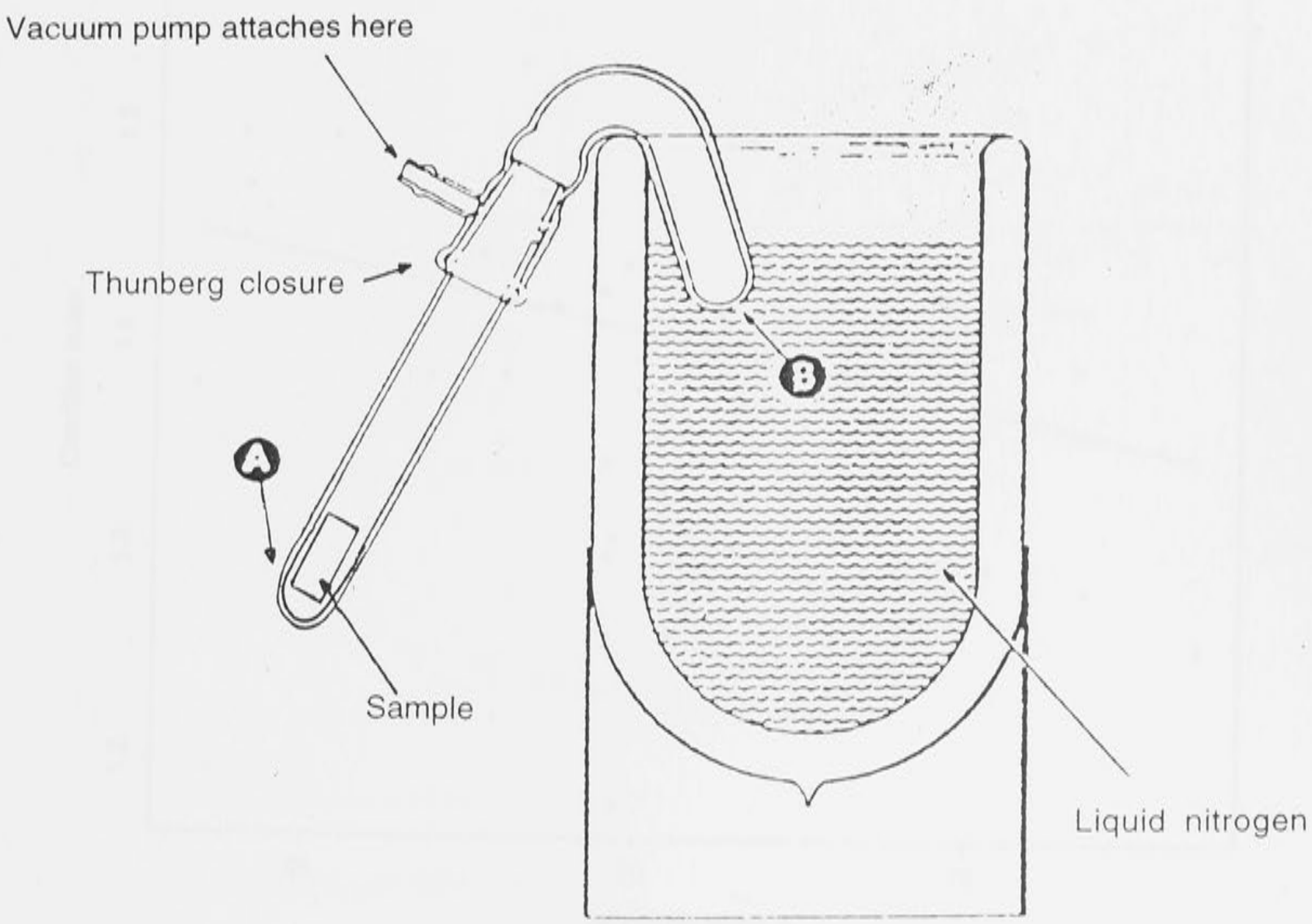


Figure 6.2: Relationship between condition index and total body water composition of *T. caninus* at Cambarville, Victoria; p=0.08 (N=52). Data were analysed using mixed models as there were repeat measures on individuals from two different seasons (winter and spring).

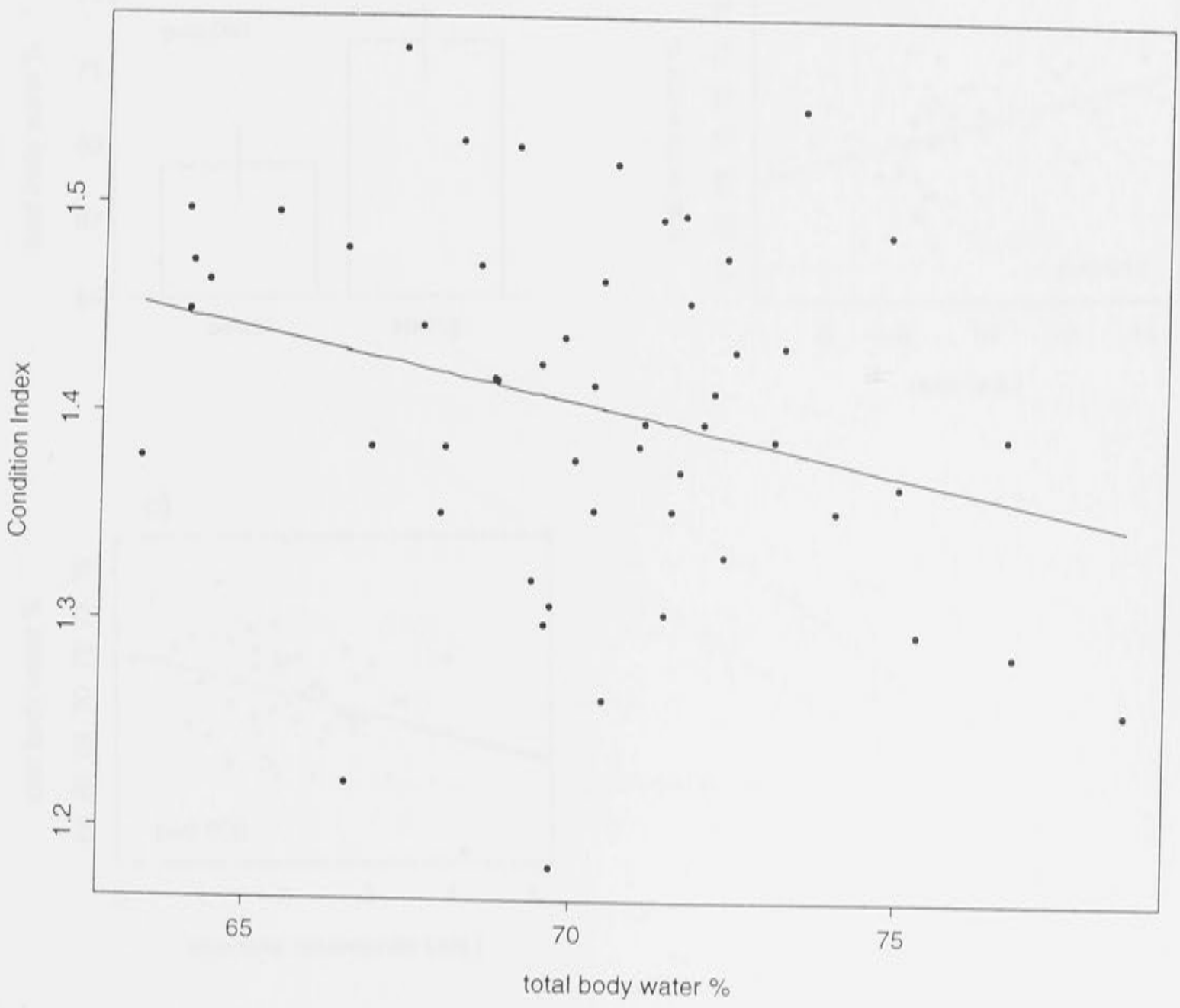
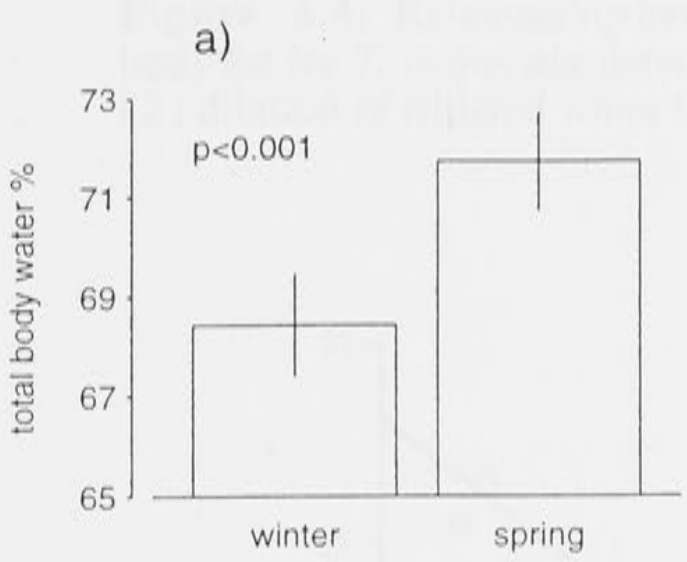
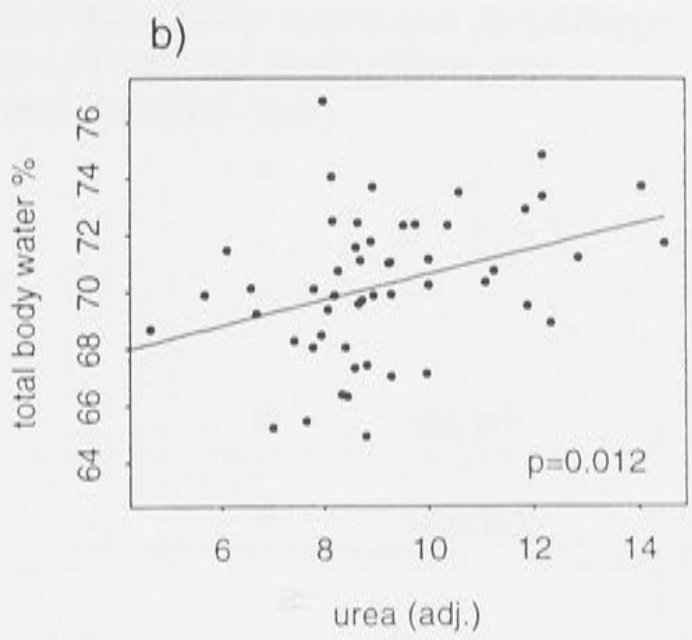


Figure 6.3: Added variable plots for significant explanatory variables in a statistical model examining variation in total body water composition of *T. caninus* in winter and spring at Cambarville, Victoria (values for each variable are adjusted for the presence of the other explanatory variables): **a)** effect of season (mean ± SE), **b)** relationship between total body water composition and serum urea levels, **c)** relationship between total body water composition and absolute neutrophils.





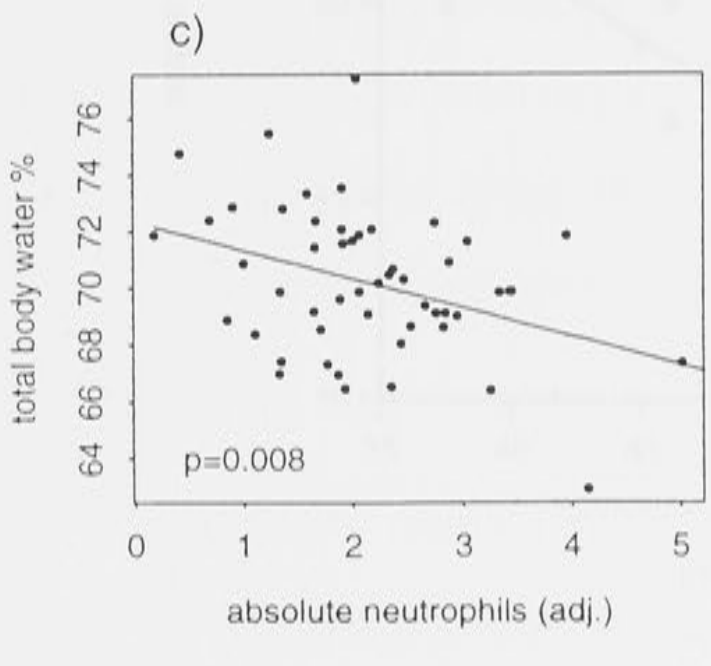
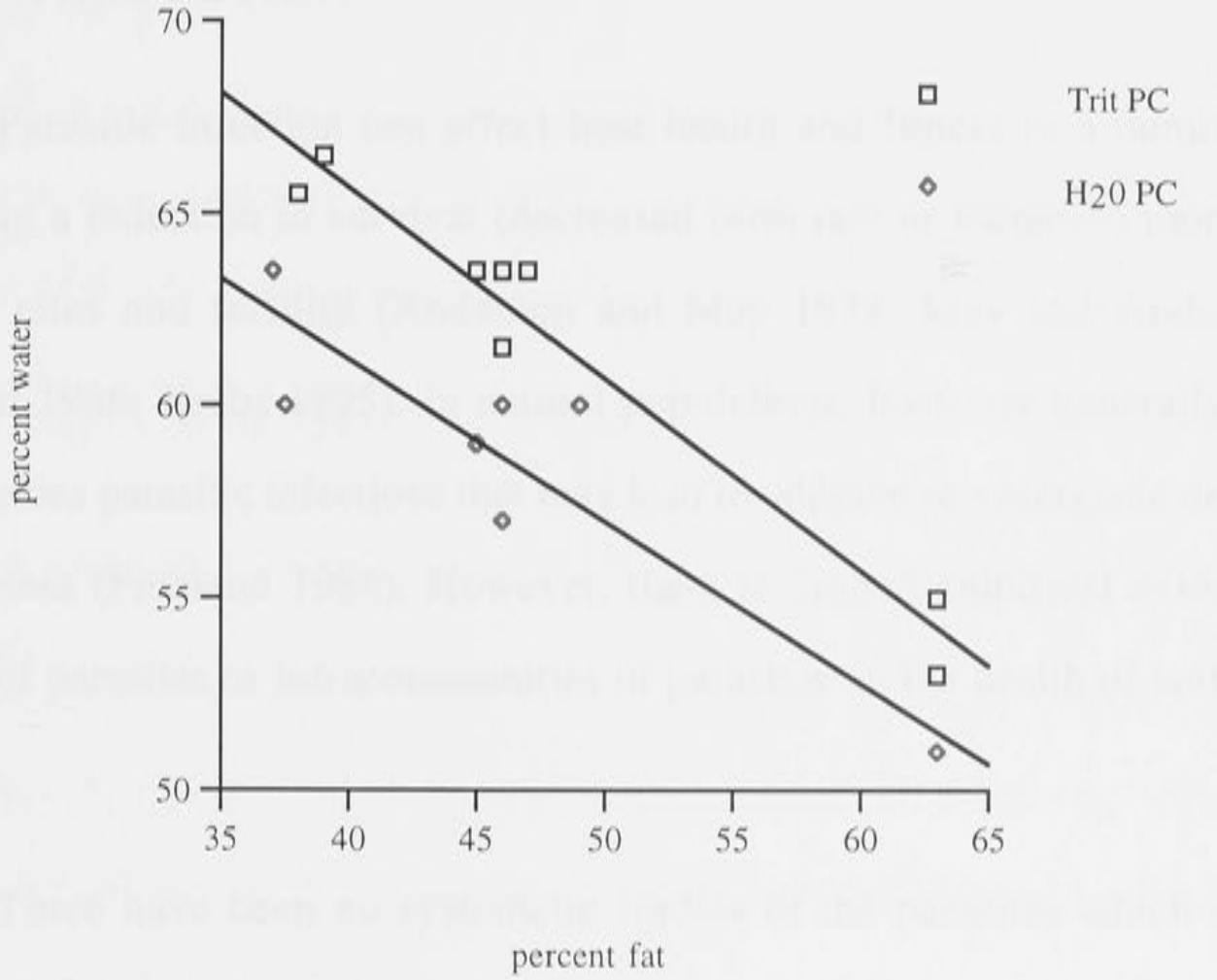


Figure 6.4: Relationship between percentage body water and percentage body fat for *T. vulpecula* determined by (**1**) carcase desiccation, and, (**2**) dilution of tritiated water (Cowan, unpublished data)



Trit PC = water content of possums estimated by tritiated water

H_20 PC = water content estimated by carcase desiccation

CHAPTER 7

PARASITES OF THE MOUNTAIN BRUSHTAIL POSSUM, *TRICHOSURUS CANINUS:*

DISTRIBUTION, ABUNDANCE AND STRUCTURE OF COMMUNITIES OF PARASITES

7.1 INTRODUCTION

Parasitic infection can affect host health and fitness in a number of ways including a reduction in survival (decreased birth rate or increased mortality rate), growth rates and fertility (Anderson and May 1978; May and Anderson 1978; Freeland 1986; Krebs 1995). In natural populations, hosts are generally subject to multispecies parasitic infections that may lead to additive or synergistic depression of host fitness (Freeland 1986). However, there is limited empirical evidence of the effects of parasites or infracommunities of parasites on the health of vertebrate host animals.

There have been no systematic studies of the parasites which occur in *T. caninus* across its geographic range. Given this, a study was undertaken to examine the infracommunities of parasites which occur in *T. caninus* at seven study sites, and the effects of these communities on the health and fitness of the host. The broad aims of this study were:- (**1**) to determine whether there was a detectable effect of the prevalence or intensity of infection with any individual species or groups of parasite taxa on the health of *T. caninus*, in terms of haematological and serum biochemical values, and, (**2**) to examine the structure of component communities of parasites of *T. caninus* at a number of sites across the host's distribution, to gain a better understanding of the patterns of occurrence of parasites in this host, and to examine

the pathological changes associated with parasitic infection. Specifically the following questions were addressed:-

(1) Are there differences in the component communities of helminths and ectoparasites of *T. caninus* between biogeographic regions; including differences in species richness, prevalence and intensity of parasitic infection?

(2) Is there a relationship between the prevalence or intensity of infection with helminths or ectoparasites and the age or sex of the host?

(3) Is there evidence of patterns of association among helminth and/or ectoparasite species?

(4) Does the presence or intensity of infection with individual parasite species or groups of parasites affect the health or condition of the host?

(5) Are there any pathological changes associated with the presence or intensity of infection with individual parasite species?

7.2 DEFINITIONS OF ECOLOGICAL TERMS IN PARASITOLOGY

A range of parasitological terms were used in this study and the definitions of these are:-

Prevalence (%) : the number of individuals of a host species infected with a particular parasite species divided by the number of hosts examined (Margolis *et al.* 1982).

Intensity: the number of individuals of a particular parasite species in each infected host (Margolis *et al.* 1982).

Mean intensity: the total number of individuals of a particular parasite species in a sample of a host species divided by the number of infected individuals of the host species in the sample (Margolis *et al.* 1982).

Abundance: the total number of individuals of a particular parasite species in a sample of hosts divided by the total number of individuals of the host species examined

(infected and uninfected) (= mean number of individuals of a particular parasite species per host examined) (Margolis *et al.* 1982).

Infracommunity: all individuals of all parasite species within an individual host (Pence *et al.* 1983).

Component community: all of the infracommunities in a population of hosts (Esch *et al.* 1975)

Compound community: all individuals of all parasite species in an ecosystem (eg. habitat patch), including those in the definitive host, intermediate hosts and free-living stages (Holmes and Price 1986).

Species richness: the number of species forming the parasite component community in a population of hosts (Poulin 1996).

7.3 METHODS AND PROCEDURES

7.3.1 Study sites, trapping, handling and sampling procedures

T. caninus was trapped at seven sites across the geographic range of the species (see Fig 3.1). In addition, a single animal from Murwillumbah in north-eastern NSW, that was presented as a fresh road-kill was included. Only adult animals (age-class ≥ 3) were included in this study. Each animal was sedated (see Chapter 3.3; Appendix 2) and blood was collected for haematological and serum biochemical analyses (see Chapter 3.5 and 3.6), as well as for the detection of microfilariae (Chapter 3.9). Blood smears were examined for the presence of blood parasites. Arthropod ectoparasites (fleas, ticks and mites) and faecal samples were also collected (Chapter 3.7 and 3.8.1).

A detailed post mortem examination was completed for a subsample of animals from each population of *T. caninus* that was sampled (see Chapter 3.10). Sample sizes of *T. caninus* were small in this aspect of the study due to difficulties in obtaining

permits to euthanase protected species in Australia. In addition, four serial faecal samples, each of approximately 10g, were collected from the distal rectum and colon of each of 12 necropsied animals and preserved in 10% formalin for faecal egg counts.

7.3.2 Statistical analyses

In studies of communities of parasites, each individual host provides a replicate habitat for parasites (Price 1990; Pence *et al.* 1983; Holmes 1987). However, much genetic and physiological variation may exist within and between host populations, such as variability due to host size, sex, age or immunological competence (Holmes and Price 1986). Where possible, these potentially confounding factors were minimised by stratifying the subsequent statistical analysis. However, when working with natural populations, some characteristics cannot be measured or accounted for, such as innate resistance to infection (Sousa 1994).

The data generated for this study were often multivariate and/or multidimensional. Statistical models were used to summarise many of the relationships examined. Where sample sizes of *T. caninus* were small, descriptive statistical methods were used (eg. principal coordinates analysis) and any other statistical tests applied were intrinsically adjusted to take account of the small sample size.

To determine whether faecal egg counts were a good representation of intensity of parasitic infection, the relationship between faecal egg counts and post mortem counts of intestinal parasites was examined. Given that *T. caninus* was sampled from several sites across its geographic range, it was necessary to include the variable "site" in the model as a fixed effect, so mixed models were used (Chapter 3.12.5; Engel 1990).

Temporal variation in the shedding of parasite eggs in the faeces was examined. Faecal egg counts were completed from four serial segments of gut from the terminal rectum and colon of 12 of the necropsied animals. Given that there were

both within and between animal observations, mixed models were used to analyse the data (Chapter 3.12.5; Engel 1990).

Patterns of occurrence of the various species of helminths, mites and fleas recorded from *T. caninus*, were examined using principle coordinates analysis (PCO), as data were multidimensional (see Chapter 3.12.6; Krzanowski 1988). In defining the distribution of a parasite species, the conjoint absence of a parasite species at paired sites was considered to be equally important as the conjoint presence of that parasite species. On this basis, a simple matching coefficient of similarity was used. When significant distributional patterns occurred, the variability in the first two principal coordinates was examined for effects of site and host sex and age-class using linear regression.

Patterns of co-occurrence among helminth and ectoparasite species were examined using PCO analysis. In examining patterns of co-occurrence of parasite species, only the conjoint presence of species was considered important. On this basis, Jaccard's coefficient, which gives weight to conjoint presence of species only, was used. When examining presence/absence data for groups of species that regularly co-occur, common and rare species should not be combined, as their co-occurrences cannot constitute a significant proportion of the occurrences of the common species (Pence *et al.* 1983). Therefore, parasite species with a prevalence of <10% were excluded from the analyses.

The prevalence of each tick species was examined to determine whether there was a significant effect of site, sex or age-class on the distribution of each tick species. Given the binary nature of the response variable (presence or absence of a species), logistic regression analysis (see Chapter 3.12.3; Collett 1991) was used.

The intensity of host infection with each parasite species was examined to determine whether there were significant differences between sites, sexes and age-classes of *T. caninus*. Large numbers of parasites were often recorded, so parasite

intensities were log transformed [log(x+1)]. Linear regression (see Chapter 3.12.2; Weisberg 1980) was used to examine the variation in the intensity of infection with various parasite species. Only those parasite species with a prevalence >30% were included in the analyses and zero occurrences were excluded. These restrictions were applied to satisfy the distributional assumptions of the statistical model because of the large number of zero observations in the data.

Variation in the species richness of parasite taxa that occurred in *T. caninus* across its geographic range was examined. The data comprised counts of parasite species, so Poisson regression analysis (see Chapter 3.12.4; McCullagh and Neldner 1989) was used to examine the effects of site, host sex and host age-class on variation in species richness. For analysis of arthropod ectoparasites, initially fleas, ticks and mites were divided into separate groups, and the species richness between different sites was compared. Species counts were then summed over these groups and the diversity of the collected ectoparasites over the range of *T. caninus* was examined.

Finally, a range of blood variables was examined using linear regression to determine whether there was a significant effect of the presence or intensity of infection with various helminth and ectoparasite species. First, data were examined to test whether the presence of a parasite species had a significant effect on blood values. Then, given that a particular parasite species was present (ie. zero values excluded), the effect of intensity of parasitic infection on blood values was examined.

7.4 RESULTS

7.4.1 Parasite fauna

A total of 104 adult *T. caninus* was captured from seven sites across the geographic range of the species (see Table 4.6). Of these, 26 animals (see Table 7.1) were euthanased for a detailed post mortem and parasitological examination (Chapter 3.10). The prevalence, intensity and abundance of each of the helminth and ectoparasite species that were recorded from *T. caninus* at each study site, are

presented in Table 7.2 and 7.3. The overall prevalence of each helminth species is shown in Fig 7.1. The filarioid nematode, *Breinlia trichosuri* was recorded only in Bulburin State Forest, central Queensland, from one *T. caninus*. New host records were established for the mite species *Murichirus anabiotus* and *Cheyletiella parasitivorax* and the fleas, *Acanthopsylla rothschildi rothschildi, Choristopsylla ochi, Bibikovana iridis* and *Stephanocircus dasyuri*. No blood parasites were detected in the stained blood smears. However, using a blood concentration technique (Chapter 3.9), sheathed microfilariae of *Sprattia venacavincola* were detected in the blood of *T. caninus* from Byrangery Reserve, the Conondale Ranges and Barrington Tops (Table 7.4). Oocysts of an undescribed species from the protozoan genus, *Eimeria*, were detected in the faeces of a number of animals from all study sites except Whian Whian State Forest (Table 7.4). All helminth and ectoparasite species recorded from *T. caninus* in this study exhibited patterns of overdispersion (ie. most hosts carried few or no parasites, whilst a few individuals carried heavy burdens of parasites).

7.4.2 Gross pathological changes associated with parasitic infection

Patchiness and thinning of the fur over the haunches (rump wear) was noted in a small proportion of *T. caninus* at all sites except Cambarville. Tick bite lesions were evident on animals from all sites and were common around the ventral aspect of the neck and beneath the mandible, as well as on the ears. Mild oedema was often detected in association with attachment or recent detachment of ticks and some tick bite wounds appeared to be infected.

There were few gross pathological changes associated with the presence of helminth parasites except the ascaridoid nematode *Ophidascaris robertsi*. Third stage larvae of *O. robertsi* were encapsulated in fibrous tissue beneath the capsular surface of the liver and throughout the liver parenchyma. Each fibrous nodule contained a single larva. Histopathological changes associated with parasitic infection will be reported elsewhere (see Chapter 8).

7.4.3 Faecal egg counts and intensity of endoparasite infection

Mixed models and linear regression were used to examine the relationship between log transformed faecal egg counts [log(x+1)] and log transformed counts of intestinal parasites (*Paraustrostrongylus trichosuri, Parastrongyloides trichosuri* and *Adelonema trichosuri*) from necropsied animals (Fig 7.2a). Diagnostic plots indicated that zero values in the data had high influence on the fit of the model. On this basis, those animals which had zero values for parasite burdens and faecal egg counts were removed from the analysis. Subsequent analysis indicated that there was no significant relationship between faecal egg count and actual parasite burden (p=0.2; Fig 7.2b).

Serial faecal egg counts from the rectum and colon of individual animals were analysed using mixed models to determine whether there was any evidence of temporal variation in the shedding of parasite eggs in faeces. As the variation within animals was very small and given that variable estimates were not significantly different when the analysis was repeated using ANOVA, means were derived from the ANOVA results (see Fig 7.3). The log of the faecal egg count [log (x+1)] was significantly higher in the most distal segment of gut than the other segments (Fig 7.3).

7.4.4 Prevalence and patterns of occurrence of parasites in *T. caninus*

7.4.4.1 Helminths

Analyses of the helminth fauna of *T. caninus* were based on a limited dataset of 26 animals collected across the distribution of the host species. A total of eight species of nematodes and one cestode was recorded from *T. caninus* over the seven study sites (Table 7.2). PCO analysis was completed to assess patterns of similarity in the occurrence (presence/absence) of infracommunities of helminths at the site level. The first two principal coordinates (PCO 1 and PCO2) accounted for 64% of the variance in the data.

Linear regression was completed for the first two principal coordinates to determine whether there was a significant effect of site, sex or age-class on the distribution of helminths in *T. caninus*. Analysis of PCO1 indicated that the patterns of occurrence of helminth species were similar in:- (**1**) Victoria (Cambarville, Bellbird) and northern NSW (Whian Whian State Forest, Byrangery Reserve, Murwillumbah), and also in, (**2**) central NSW (Barrington Tops) and Queensland (Conondale Ranges and Bulburin State Forest) (Fig 7.4). However these two groupings were significantly different from each other ($p<0.001$; see Fig 7.5). Central NSW and Queensland were similar due to the presence of *O. robertsi* and *Marsupostrongylus minesi* and the absence of *S. venacavincola* (Fig 7.4). *Gongylonema* sp. was detected both in Victoria and northern NSW, but not elsewhere (Fig 7.4). *Paraustrostrongylus trichosuri* was detected at high prevalences in all regions except Victoria. *M. minesi* was highly prevalent in Queensland and southern NSW, but was uncommon or absent in northern NSW and Victoria (Fig 7.4). The estimates of regression coefficients and standard errors for this statistical model are given in Table 7.5. There was also a significant effect of site on PCO2 ($p<0.001$) (Table 7.6). In the post mortem subsample of animals used in these analyses, *S. venacavincola* was recorded only from northern NSW. There was no effect of host sex or age-class on the distribution of helminths among host animals.

7.4.4.2 Ticks

Logistic regression analysis was completed to determine the patterns of occurrence of each species of tick that was recorded from *T. caninus* captured at the seven study sites. There was a significant effect of site on the occurrence of both *Ixodes holocyclus* and *I. tasmani* (Tables 7.7 and 7.8). *I. holocyclus* was not detected in *T. caninus* at Cambarville in central Victoria and *I. tasmani* was absent from Bulburin State Forest in central Queensland.

7.4.4.3 Fleas

Principal coordinates analysis (PCO) was used to assess patterns of occurrence among species of fleas recorded from *T. caninus* at the seven sites; the first two principal coordinates accounted for 87% of the variance in the data. A plot of the scores of PCO1 and PCO2 demonstrated clear differentiation of the flea infracommunities in *T. caninus* (see Fig 7.6). These groupings were:- (**1**) those animals which carried no fleas and those that carried only *S. dasyuri*, *B. iridis* or *C. ochi*; (**2**) animals in Victoria, which were distinguishable by the presence of the flea *A. rothschildi rothschildi*; and, (**3**) animals from NSW and Queensland, which were distinguishable by the absence of *A. rothschildi rothschildi*.

7.4.4.4 Mites

Principal coordinates analysis also was completed to assess patterns of occurrence among infracommunities of mites recorded from *T. caninus*. The first two principal coordinates accounted for 67% of the variance in the data. Linear regression was used for PCO1 and PCO2 to examine the effects of site and sex on the data. Significant explanatory variables in a statistical model examining variation in PCO1 were site, sex and a site by sex interaction. Closer examination of the data revealed that the site by sex interaction was explained by a difference between the sexes at one site only (Bellbird in eastern Victoria). An equally robust, more parsimonious model was achieved by re-labelling the sites to form a new variable "group" including variables for each site and a separate variable for male and female *T. caninus* at Bellbird (Table 7.9). The significant variables in the final model were sex ($p<0.001$) and group ($p<0.001$).

The prevalences of the different species of mites at the seven sites across the geographic range of *T. caninus* are shown in Fig 7.7, and for the different sexes in Fig 7.8. The prevalence of all mite species was lower in female *T. caninus* than in male animals ($p<0.001$). The infracommunities of mites at Whian Whian, Byrangery

and in Bellbird females were significantly different from the others ($p<0.001$). At Whian Whian, Byrangery and in Bellbird females, *M. anabiotus* was absent and the prevalences of *Atellana papilio* and *Petrogalochirus dycei* were low (<20%). In addition, the prevalences of the more common mite species, *Trichosurolaelaps crassipes, T. dixous, Haemolaelaps penelope* and *H. sisyphus* were also substantially lower than at the other sites.

Linear regression of the second principal coordinate (PCO2) revealed that "site" was the only significant explanatory variable in the resulting statistical model ($p<0.001$). The regression coefficients and standard errors for this statistical model are shown in Table 7.10. Mite infracommunities from *T. caninus* in Victoria were significantly different to those recorded from animals in Queensland. This difference was due to the lower prevalences of *M. anabiotus* and *C. parasitivorax* among Queensland animals compared with those from Victoria.

7.4.5 Intensity of parasitic infection in *T. caninus*

To investigate potential relationships between the intensity of parasitic infection and possible explanatory variables, linear regression analysis was used. Only parasites with a prevalence greater than 30% were included in these analyses and zero values were excluded. There was a significant effect of site on the intensity of infection with the intestinal nematode *Paraustrostrongylus trichosuri* ($p=0.009$), with higher worm burdens recorded from animals at Cambarville and Bellbird than those at Bulburin State Forest and Barrington Tops (Fig 7.9).

Linear regression was used to examine variability in the intensity of infection with ectoparasite species. There was a significant effect of site on the intensity of infestation with the mites *T. crassipes* ($p<0.001$) and *T. dixous* ($p<0.001$), both of which were present at a lower intensity at Bellbird and Whian Whian than the other study sites (Fig 7.10a and 7.10b). The intensity of infestation with the ixodid tick *I.*

tasmani was found to be significantly higher at Whian Whian than at any of the other sites (p=0.02) (Fig 7.11).

7.4.6 Species richness

The species richness of helminths was examined using Poisson regression analysis. A significant effect of site on species richness was detected (p=0.03) (Fig 7.12). The number of helminth species recorded from *T. caninus* was higher in central NSW (Barrington Tops) and Queensland (Conondale Ranges and Bulburin State Forest) than from those animals in Victoria and northern NSW (p=0.03).

Poisson regression analysis also was completed to compare the species richness of ectoparasites between sites, as well as between male and female *T. caninus*. A higher number of tick species was detected for *T. caninus* at Whian Whian State Forest and Byrangery Reserve in northeastern NSW than at the other sites (p=0.004) (Fig 7.13). Species richness of fleas was also found to be higher at Whian Whian (p=0.01) (Fig 7.14). Analyses of species richness of mites produced significant site and sex main effects, as well as a site by sex interaction at Bellbird. In general, female *T. caninus* carried a lower number of mite species than male animals (p<0.001). A significantly lower number of mite species was recorded from animals at Whian Whian State Forest and Byrangery Reserve than at the other sites (p<0.001). In addition, a significantly lower number of species of mites was detected on female *T. caninus* at Bellbird than from male animals at this site (p<0.001). The species richness of all ectoparasite species combined was not significantly different between sites or sexes.

7.4.7 Patterns of co-occurrence among helminth and ectoparasite species

Principal coordinates analysis using Jaccard's matching coefficient was completed for helminth species that were recorded from *T. caninus,* excluding rare species. Some loose clustering of nematodes was apparent (Fig 7.15):

Paraustrostrongylus trichosuri, Parastrongyloides trichosuri, A. trichosuri and *M. minesi* tended to occur together. *O. robertsi* and *Bertiella trichosuri* were represented by isolated points in the two dimensional data matrix (see Fig 7.15), indicating that neither species co-occurred with any other helminth species.

PCO analysis of the ectoparasite species of *T. caninus*, revealed no distinct patterns of co-occurrence among species (Fig 7.16). Species of mites were somewhat clustered, however, this probably reflects their abundance and ubiquity. Rare species of mites and fleas were excluded from these analyses.

The combination of helminth and ectoparasite species, excluding rare species, showed no distinct patterns of co-occurrence amongst species (Fig 7.17).

7.4.8 Blood analyses and parasite intensity

The blood variables for which significant effects were detected due the presence and intensities of particular parasite species are given in Tables 7.11 and 7.12 respectively. The log of the white cell count (WCC) was significantly affected by both the State in which the animal was resident and the presence of *I. holocyclus* (Figs 7.18a and 7.18b respectively). There was also a significant positive relationship between the log of the white cell count and the intensity of infestation with *I. holocyclus* (Figs 7.19a and 7.19b). A positive relationship was also found between the log(neutrophils) and the intensity of infestation with this tick species (Figs 7.20a and 7.20b). Total serum protein levels were significantly affected by State and the presence of *I. trichosuri* (Fig 7.21a and 7.21b), as well as by the intensity of infestation with this tick (Fig 7.22a and 7.22b). Serum protein levels were higher in those animals infested with *I. trichosuri*.

7.5 DISCUSSION

7.5.1 Distribution of parasites in host individuals and gross pathological changes associated with helminth infection

The distribution of helminths and ectoparasites in populations of *T. caninus* was generally overdispersed (aggregated), ie. most hosts carried few or no parasites whilst a few individuals carried heavy burdens of parasites. This appears to be typical of natural populations (Bradley 1972; Anderson and May 1978; Wakelin 1985; Halvorsen 1986; Shaw and Dobson 1996). Factors which may contribute to over- and underdispersion of parasites in host populations include:- **(1)** heterogeneity between hosts, such as genetic variability in susceptibility to disease, and differences in individual natural resistance and acquired immunity (ie. immunological variation associated with past exposure to infection), **(2)** density-dependent (crowding) constraints within each host at the infracommunity level, which affect the rates at which parasites establish, survive and reproduce (Wakelin 1985; Wallace and Pence 1986; Shaw and Dobson 1996), and, **(3)** variation in the acquisition of infective stages of parasites by hosts, which is affected by the feeding behaviour of the host, the distribution and density of the infective stages and the survival and infectivity of the infective stages (Keymer and Anderson 1979). Assuming that host mortality increases with increasing parasite burdens, Anderson and May (1978) showed that parasites with an aggregated distribution may influence host population growth rate. For *T. caninus*, the relationship between parasite burdens and host mortalities is unknown. This relationship is unknown for most natural populations due to the difficulty in detecting ill or dead animals in the field, as they may die in inaccessible places or be rapidly removed by predators and scavengers (McCallum and Dobson 1995). Given this, controlled field-based experiments to manipulate parasite burdens (Krebs 1995) are required to determine the effects of parasites on the survival of *T. caninus*.

There were few gross pathological changes associated with the presence of helminth parasites in *T. caninus*. For example, *Paraustrostrongylus trichosuri* is generally found tightly coiled around the villi of the proximal small intestine (Mawson 1973; Smales and Mawson 1978; Presidente 1984, Presidente *et al.* 1982); however, even when present at high intensities in *T. caninus* in this study, no grossly visible pathological changes were evident. The other parasite observed in the proximal small intestine, *Parastrongyloides trichosuri*, was never present at high intensities. Although large burdens of *A. trichosuri* were occasionally detected, this parasite is found in the caecal contents and does not appear to have any pathological effects. The metastrongyloid nematode, *M. minesi,* was recorded from *T. caninus* at several study sites (see Table 7.3), however no gross pathological changes were evident in the lungs of infected animals. The anoplocephalid cestode, *Bertiella trichosuri*, was present only at low intensities and did not appear to cause any gross pathological changes in host tissues.

O. robertsi was the only helminth parasite with which there were associated gross pathological changes. Presidente *et al.* (1982) reported a finding of 216 *O. robertsi* larvae in the liver of an adult female *T. caninus* at Clouds Creek. Despite the high numbers of parasites, comprising 1/3 of the liver and weighing 28 grams, this animal was in good condition and carrying a pouch young.

Some helminths, such as *Breinlia trichosuri* and *S. venacavincola,* were recorded too infrequently to determine their pathological effects on the host. In another study, Presidente *et al.* (1982) failed to find adults of *S. venacavincola* in *T. caninus* at Clouds Creek, in northeastern NSW, but the detected sheathed microfilariae of this parasite in blood smears from 25% of *T. caninus*. These authors also reported nodules in the spleen which represented granulomatous reactions to the presence of sequestered microfilariae.

7.5.2 Faecal egg counts and intensity of helminth infections

In the initial analysis of faecal egg counts and intestinal nematodes, a significant relationship was found (see Fig 7.2a). However the zero values for faecal egg count had high residuals and high influence on the relationship, thereby rendering the analysis unsound. Although it is possible to obtain a zero faecal egg count in the presence of significant numbers of nematodes, in this example a statistically sound relationship could not be achieved with the zero values included. Therefore the analysis was repeated excluding data points with zero values for faecal egg count, and there was no significant relationship (Fig 7.2b). This suggests that faecal egg count may not be a good indicator of intensity of gastrointestinal helminth infection in *T. caninus*. However, these findings may been partly due to the limited sample size (N = 26) in this study. Studies of other species with larger sample sizes have shown that faecal egg count may correlate well with the intensity of parasites in the gastrointestinal tract (Anderson and May 1991; Gregory 1991; Stear *et al.* 1995).

Faecal egg counts in *T. caninus* also may have been influenced by variation in the fecundity of nematode species. In a study of the epidemiology of parasitic infections in the eastern grey kangaroo, *Macropus giganteus*, minimum egg counts occurred at a time of year when several nematode species were at their most abundant, suggesting that those more abundant species were not particularly fecund (Arundel *et al.* 1990).

The analysis of egg counts from faecal samples collected sequentially from the distal rectum and colon of *T. caninus*, showed that egg counts were significantly higher in the most distal segment of the gut (Fig 7.2). Faeces collected from the terminal section of the gut also appeared to be substantially drier than faeces collected from more proximal sections. This appears to reflect a concentration effect, induced by dehydration of faeces, rather than a temporal variation in the shedding of parasite eggs. Given this, faecal egg counts completed from a random sample of scats collected from the bottom of the cage in which each animal was trapped, should provide a

useful tool for comparisons between host individuals of the numbers of eggs shed by intestinal parasites. Repeatability of faecal egg counts in individuals was not tested. However, studies on sheep grazing pastures infected with *Haemonchus contortus* have shown that the same animals tended to have the highest faecal egg counts on each occasion that they were measured (Barger and Dash 1987).

7.5.3 Sample size limitations, and effects of host age and sex on prevalence, species richness and intensity of infection with parasites in *T. caninus*

This study was based on samples of helminths and ectoparasites collected from *T. caninus* at seven sites which represent limited samples within the host's total geographic range. Therefore, it is likely that some parasite species which occur in this host were not recorded. The prevalence of some parasites recorded in this study, particularly helminths, may have been under-estimated by the small sample size from each site. For example, although the post mortem subsample recorded *S. venacavincola* from only one animal in northeastern NSW, testing for this parasite by a concentration technique in a larger sample of live animals (N=104) (see Chapter 3.9), revealed a higher prevalence and wider distribution of this parasite (Table 7.4). Given this, zero prevalences may be interpreted in two ways:- **(1)** there is a true prevalence of zero, or, **(2)** a low prevalence exists, which may be detected with a larger sample size (Gregory and Blackburn 1991). Prevalence data should therefore be interpreted giving due consideration to the sample size (Gregory and Blackburn 1991). Estimates of parasite species diversity also may be influenced by host sample size: as the host sample size increases, the diversity of parasite species recorded generally also increases (Gregory and Blackburn 1991).

There was no effect of host age-class on the distribution of helminths or arthropod ectoparasites in *T. caninus* at the seven study sites. This differs from other hosts in which some parasites may vary depending on host age. For example, *Dirofilaria immitis* was significantly more prevalent and present at higher intensities in

older red wolves, *Canis rufus*; (Custer and Pence 1981). More commonly, helminth prevalence and intensity of infection decreases with age (Pence and Windberg 1984). This may be due to:- **(1)** reduced susceptibility to infection through previous exposure and development of the immune response; and, **(2)** changes in diet, behaviour in older animals (Pence and Windberg 1984). In this study of *T. caninus*, only adult animals were examined (≥ age-class 3) and no distinct patterns of occurrence of parasites were detected for different age-classes of adults.

7.5.4 Structure of communities of parasites

Studies of the structure of communities of parasites may provide information on interactions between parasite species and the evolutionary ecology of host-parasite systems (Holmes 1987). Parasite community structure may be examined at the infracommunity, component community and compound community levels. Identification of the structure of communities of parasites within host individuals and populations may facilitate an examination of the conditions which give rise to community organisation (Holmes 1987).

7.5.4.1 Parasite infracommunities

Many factors may influence parasite infracommunity structure, including:- **(1)** physiological and biological processes that influence host exposure to parasites, such as host, diet and foraging strategies (Keymer and Anderson 1979; Kennedy *et al.* 1986; Guégan and Hugeny 1994) and host vagility (Freeland 1986; Kennedy *et al.* 1986); **(2)** host and parasite factors which influence the establishment and viability of a parasitic infection, such as host age, sex, physiological condition, immune defences, parasite virulence (Freeland 1986; McCallum 1994) and stress (Esch *et al.* 1975); **(3)** direct and indirect interactions in mixed species infections of parasites (Sousa 1994), and, **(4)** the complexity of the host gastrointestinal tract (ie. a more complex gut may provide more niches for different parasite species) (Kennedy *et al.* 1986; Beveridge and Spratt 1996).

Holmes and Price (1986) recognised two types of parasite infracommunities: interactive and isolationist. Interactive infracommunities were defined as those comprised of parasite species with a high probability of being transmitted between hosts (ie. high colonising ability), generally present at high intensities and therefore likely to be interacting through inter- and intraspecific competition. Alternatively, isolationist infracommunities were seen as those communities of parasite species with a low probability of transmission, present generally at low intensity and with a low probability of interaction between species (Holmes and Price 1986). In *T. caninus*, helminth infracommunities tended to be species poor (see Table 7.3), with several vacant niches present in most host individuals. Unoccupied niches frequently were evident in the gastrointestinal tract of the host; including the lower proximal and distal small intestine in which *Bertiella trichosuri* was infrequently detected, and the stomach, from which no parasites were collected. This may reflect either a failure of any parasite species to colonise these sections of the gastrointestinal tract or an elimination by host defences of any parasites which previously occupied these niches (Freeland 1986).

In the proximal small intestine of several host individuals, *Paraustrostrongylus trichosuri* and *Parastrongyloides trichosuri* occurred together. However, the prevalence and intensity of infection with either species did not appear to be altered by the presence of the other (ie. was not density dependent). There was no other evidence of interaction between species in niches occurring in this host. The distribution and intensity of infection with helminth species in *T. caninus* suggested that helminth infracommunities had characteristics tending towards an isolationist structure. Freeland (1986) suggested that parasite species within communities which were not interactive tended to be overdispersed, were often more virulent and may be more likely to contribute to fluctuations in populations of hosts and parasites. Further research, in the form of field-based experiments, is required to determine the potential of parasite infracommunities in *T. caninus* for regulation of host populations.

Another approach to examining helminth infracommunity structure is to define groups of species within communities (Holmes 1987). Hanski (1982) proposed that communities may consist of a number of core species (those which occur at high prevalence and abundance) and satellite species (those found in a small proportion of individuals generally in low numbers). Core species are often host specific and hence have probably co-evolved with their hosts (Holmes 1987), whereas satellite species are often those which have made a transition to another host from related host species (see Beveridge and Spratt 1996). In order to classify the species which form infracommunities in *T. caninus*, the definitions established by Fedynich and Pence (1994) were followed: core species recorded from parasite populations of *T. caninus* were those with prevalences >70%, satellite species < 20% prevalence and intermediate species between 20-70% prevalence. Difficulties may arise in determining the core species when numerous species occur at intermediate prevalences (Holmes 1987). Of the nine helminth species recorded across the range of *T. caninus*, there was only one core species *(Paraustrostrongylus trichosuri*: 92% prevalence [23/25]); 44% (4/9) of species were intermediate and 44% (4/9) were satellite species (see Fig 7.1). The presence of a majority of intermediate species in this host indicates that Hanski's (1982) theory could not be easily applied to helminth infracommunities of *T. caninus*.

7.5.4.2 Parasite component communities

Parasite community structure also may be studied at the component community level, to examine what factors contribute to the species richness of parasites which occur in a host species (Holmes and Price 1986). Variation in parasite component communities, species richness and intensity of parasitic infection is often observed across the geographic range of a host species and this may be partly due to habitat diversity (Pence 1990). Parasites require:- (**1**) conditions that allow the transmission between hosts, and/or, (**2**) suitable conditions for free-living stages of the lifecycle,

and/or, (**3**) suitable intermediate hosts (Holmes and Price 1986). All of these factors may be influenced by climatic and habitat variability.

Regional biogeographic and habitat differences may have contributed to the patterns of occurrence and species diversity in the parasites recorded from *T. caninus*. For example, the distribution of the ixodid tick, *I. holocyclus*, relies on appropriate climatic conditions for the hatching of eggs and the survival and moulting of instars, as well as the availability of suitable hosts (Doube 1974; Heath 1979, 1981; Aeschlimann 1991).

The distributions of intermediate hosts are also limited by climatic conditions, which consequently influences the distribution and abundance of those species with indirect life-cycles (Holmes and Price 1986). *S. venacavincola* and *Breinlia trichosuri* were only recorded from *T. caninus* in northeastern NSW and southeastern Queensland and it appears that appropriate intermediate hosts (which are currently unknown) may occur only in those States. The metastrongyloid nematode, *M. minesi,* also may be limited by the distribution of its intermediate host, which is probably a gastropod (Spratt 1979). Alternatively, *O. robertsi* is limited by the distribution of its definitive host, the carpet python, *Morelia spilota variegata,* which is absent from the central highlands of Victoria (Lumsden *et al.* 1991), where one of the seven populations of *T. caninus* examined in this study was located and from which *O. robertsi* was therefore absent.

Species richness may be greater at higher host densities, due to the larger effective host population size which may enable more parasite species to be maintained at a local level (Price 1990). Helminth species richness in populations of *T. caninus* was relatively low when compared with other host species. For example, in a study of the carnivorous marsupial mouse, *Antechinus stuartii*, at a site in central Victoria, 14 helminth species were recorded (Beveridge and Barker 1976). Macropodid marsupials frequently are infected with large numbers of helminth species (eg. eastern grey kangaroos, *Macropus giganteus*, Beveridge and Arundel 1979; rock wallabies,

Petrogale spp., Beveridge *et al.* 1989; Proserpine rock wallaby, *Petrogale persephone*, Begg *et al.* 1995). The low species richness recorded from *T. caninus* may be associated with the diet and foraging habits of the species, as well as the characteristics of the habitat in which it is found (see Chapter 2). Species at the top of the food chain, or those which ingest a wide variety of dietary items, (particularly invertebrates, which may function as intermediate hosts for parasites) typically exhibit higher species richness in their parasite component communities (see Kennedy *et al.* 1986). For example, lesser scaup ducks, *Aythya affinis*, acquire many species of helminths by ingesting infected intermediate hosts (Bush and Holmes 1985). *T. caninus* does not actively seek invertebrates as dietary items, and is therefore exposed to few intermediate hosts, which is probably reflected in the low helminth species richness recorded for *T. caninus* in this study.

Patterns of co-occurrence or association among helminth and/or ectoparasite species may provide further information on parasite component community structure. In *T. caninus*, there was a loose tendency for *Paraustrostrongylus trichosuri, Parastrongyloides trichosuri, A. trichosuri* and *M. minesi* to co-occur (see Fig 7.15). These species are all nematodes for which *T. caninus* is the definitive host. The occurrence of *O. robertsi* and *Bertiella trichosuri* was not associated with the presence of any other species. The distribution of the definitive host for *O. robertsi*, the carpet python (*M. s. variegata*) would have been the major determinant in the patterns of distribution of this parasite. *Bertiella trichosuri* is an anoplocephalid cestode which has an indirect life-cycle (see Appendix 1: Viggers and Spratt 1995). The distribution of this parasite would have been determined by the distribution of its unknown intermediate host, which was unlikely to be related to the occurrence of any other helminth species recorded from *T. caninus* in this study. Interrelationships between common species of helminths and ectoparasites of *T. caninus* were examined, but no distinct patterns of co-occurrence of species were found.

Two main hypotheses have been developed to explain the evolutionary processes contributing to helminth component community structure. These are:- co-evolution and host-switching (see Beveridge and Spratt 1996). The co-evolution hypothesis proposes that cospeciation with the host group has produced communities of parasites that have co-evolved (ie. that parasites speciated at the same time or following speciation in the host) (Pence 1990; Beveridge and Spratt 1996). Alternatively, the host-switching hypothesis suggests that parasites may switch to an unrelated host or be "captured" by that host (Goater *et al.* 1987). Examination of the individual helminth species that occur in *T. caninus* provides some evidence for both hypotheses. Anoplocephalid cestodes of the genus *Bertiella* occur in three marsupial families and rodents (Beveridge and Spratt 1996). These families are not closely related, but do share some habitat overlap, which suggests that host-switching may have occurred (Beveridge and Spratt 1996). Representatives of the genus *Paraustrostrongylus* occur in potoroids, phalangerids, petaurids and macropodids, suggesting that this genus also has made the transition to different hosts (Beveridge and Spratt 1996). Host switching by parasites with indirect life cycles may be influenced by the type of intermediate host, eg. the gastropod intermediate hosts of metastrongyloid nematodes may be accidentally ingested by other hosts (Beveridge and Spratt 1996). Conversely, oxyurid nematodes such as *Adelonema trichosuri*, are transmitted from host to host by direct ingestion of the infective egg. The eggs of oxyurid nematodes are sticky and tend to cause irritation around the anus of the animal which stimulates grooming and therefore facilitates re-infection. This mode of transmission provides an isolating mechanism which is more likely to lead to speciation and co-evolution.

7.5.4.3 Parasite compound communities

The parasite compound community comprises all populations of all individuals of all parasite species in an ecosystem, including those in definitive hosts, intermediate hosts and free-living stages (Holmes and Price 1986). Given that little is known of the

life-cycles of most of the parasites which infect *T. caninus*, no examination was made of communities of parasites at this level.

7.5.4 Blood analyses and parasite intensity

There was a significant association between some ectoparasite species and a number of haematological and serum biochemical values of *T. caninus*. The presence and intensity of infestation with *I. holocyclus* were associated with increased white cell and neutrophil counts. The presence of *I. holocyclus* was also associated with higher serum globulin levels. Ticks have mouthparts that are adapted for cutting skin (Wakelin 1985) and *I. holocyclus* has relatively long mouthparts which penetrate about 1mm below the skin surface (Stone 1990). Feeding is preceded by the production of saliva which contains proteins that are strongly immunogenic (Wakelin 1985; Stone 1990). These proteins cause pronounced inflammation and a strong immune response, which is characterised by local infiltration of eosinophils and basophils (Wakelin 1985; Allen 1986; Stone 1990; Brossard *et al.* 1991). Local release of histamines and other inflammatory mediators from degranulating basophils and mast cells may disrupt tick feeding and cause pruritis, stimulating grooming behaviour by the host to help remove the tick (Allen 1986). *T. caninus* carried large numbers of *I. holocyclus* at some study sites (eg. Byrangery Reserve) and it is possible that chronic tick infestation in this host may give rise to inflammatory responses that are detected in the peripheral circulation, such as increased white cell and neutrophil counts (Bush 1991). A similar finding has been reported for other host species. Infestation with ticks (*Haemaphysalis humerosa, I. holocyclus* and *I. tasmani*) in juvenile northern brown bandicoots, *Isoodon macrourus*, resulted in higher white cell counts in comparison to tick-free animals of the same age, and this was attributed to either the presence of the ticks, or parasites transmitted by the ticks (Gemmell *et al.* 1991). In another study, the presence of ticks (*Argas cooleyi* and *Ornithodoros concanensis*) and the swallow bug, *Oeciacus vicarius*, in nest sites of cliff swallows, *Hirundo pyrrhonota*, was associated with higher white cell counts in

nestlings compared with those from nests that had been sprayed with an insecticide to remove ticks (Chapman and George 1991).

In some hosts, changes in the haematological and serum biochemical values have been recorded in association with helminth infections. In juvenile eastern grey kangaroos, *Macropus giganteus*, there was a strong relationship between the numbers of the pathogenic nematode *Globocephaloides trifidospicularis*, and decreasing levels of plasma protein, haemoglobin concentration and haematocrit values. In addition, this parasite caused considerable mortality in juvenile *M. giganteus* (Arundel *et al.* 1990). No significant correlation was found between presence or intensity of helminth infections and blood values of *T. caninus* in this study.

7.6 CONCLUSIONS

Although there were few gross pathological changes associated with helminth infections in *T. caninus*, it cannot be assumed that these parasites and parasite infracommunities do not have a detrimental effect on the host. Further information on the pathogenic effects of parasites can be obtained by histopathological examination of tissues from parasitised hosts (see Chapter 8).

The species richness of helminth infracommunities recorded from *T. caninus* was low, which may reflect the habitat and foraging behaviour of this host. There was evidence that parasite species comprising the infracommunities of *T. caninus* were isolationist, rather than interactive. However, this provides little information on the impacts of these parasites on the host population. In natural populations, it is difficult to assess the combined effect of multispecies parasitic infections on the fitness, competitive ability and reproductive success of the host (Freeland 1986). To determine these effects, manipulation of parasite burdens in controlled field experiments is required (Scott and Dobson 1989; Sousa 1994; Krebs 1995; McCallum and Dobson 1995). Using information derived from this study of parasite infracommunities of *T. caninus*, a field experiment of this type was completed (see Chapters 9 and 10).

Table 7.1: Number and sex of *T. caninus* necropsied from each study site

Site	Male	Female
Cambarville (Vic)	4	4
Bellbird (Vic)	1	0
Whian Whian (NSW)	1	0
Byrangery Reserve (NSW)	2	1
Conondale Ranges (Qld)	1	3
Bulburin State Forest (Qld)	3	1
Barrington Tops (NSW)	2	2
Murwillumbah (NSW)	1	0
TOTAL	15	11

Table 7.2: The helminths recorded from *T. caninus* from seven sites across the geographic range of the species

Site			C (N=8)			BB (N=1)			WW (N=1)			BR (N=3)			CD (N=4)			BSF (N=4)			BTP (N=4)			MW (N=1)		
Parasite	Overall Var	Mean	P	I	A	P	I	A	P	I	A	P	I	A	P	I	A	P	I	A	P	I	A	P	I	A
Nematoda																										
Pat	$17x10^5$	898	100	1698	1698	100	5150	5150	0	0	0	67	359	239	100	514	514	100	142	142	100	95	95	100	82	82
Pt	1443	30.3	13	43	5.4	100	60	60	0	0	0	67	120	80	100	40	40	100	49	49	75	20	14.7	100	25	25
At	$21x10^5$	492	13	81	10.1	100	4	4	0	0	0	0	0	0	100	1436	1436	75	2148	1611	75	8.3	6.3	0	0	0
Or	268.3	4.2	0	0	0	0	0	0	0	0	0	0	0	0	25	5	1.25	100	23	23	25	8	2	0	0	0
Bt	0.04	0.04	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	1	0.25	0	0	0	0	0	0
Sv	0.08	0.08	0	0	0	0	0	0	0	0	0	67	*	*	0	0	0	0	0	0	0	0	0	100	1	1
Mm	564.3	31.8	0	0	0	0	0	0	100	1	1	0	0	0	100	25.5	25.5	100	18	18	100	155	155	0	0	0
G	6.8	0.52	0	0	0	100	3	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	1	1
Cestoda																										
Bet	2.7	0.96	63	2.8	1.75	100	1	1	0	0	0	0	0	0	0	0	0	0	0	0	50	4.5	2.25	0	0	0

P = prevalence (%) = no. infected / no.examined
I = mean intensity = total helminths / no. hosts infected
A - abundance = total helminths / no.hosts examined
* - only presence/absence data collected, no counts

Sites: C=Cambarville, BB=Bellbird, WW=Whian Whian State Forest, BR=Byrangery Reserve, CD=Conondale Ranges, BSF=Bulburin State Forest, BTP=Barrington Tops, MW = Murwillumbah

Key to parasites: Pat = *Paraustrostrongylus trichosuri*; Pt = *Parastrongyloides trichosuri*; At = *Adelonema trichosuri*; Or = *Ophidascaris robertsi;* Bt = *Breinlia trichosuri*; Sv = *Sprattia venacavincola*; Mm = *Marsupostrongylus minesi*; G = *Gongylonema* sp.; Bet = *Bertiella trichosuri*

Table 7.3: The ectoparasites recorded from *T. caninus* from seven sites across the geographic range of the species

Site	Overall		Cambarville (N=33)			Bellbird (N=13)			Whian Whian (N=7)			Byrangery (N=7)			Conondales (N=13)			Bulburin (N=13)			Barrington Tops (N=18)		
Parasite	Variance	Mean	P	I	A	P	I	A	P	I	A	P	I	A	P	I	A	P	I	A	P	I	A
Ticks																							
Ih	4.0	0.6	0	0	0	8	1	0.08	71	3.2	2.3	71	7	5	15	1	0.1	23	1	0.23	17	2.7	0.4
Itr	0.7	0.4	27	1.6	0.4	31	3	0.9	29	1.5	0.4	14	1	0.1	31	1.3	0.4	15	1	0.2	11	1.5	0.2
Ita	0.8	0.4	9	1	0.1	15	2	0.9	71	3.2	2.3	43	1	0.4	31	1.5	0.5	0	0	0	28	1.2	0.3
Fleas																							
Ap	5.7	1.0	0	0	0	0	0	0	100	4.9	4.9	14	1	0.14	54	1.6	0.9	31	1.8	0.5	83	3.3	2.8
Arr	0.6	0.2	30	2	0.6	8	1	0.08	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Co	2.0	0.2	0	0	0	15	10	1.5	14	1	0.14	0	0	0	0	0	0	0	0	0	0	0	0
Bi	0.01	0.01	0	0	0	0	0	0	0	0	0	0	0	0	8	1	0.08	0	0	0	0	0	0
Sd	0.01	0.01	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	1	0.06
Mites																							
Tc	966.0	28.9	91	43.5	39.6	46	10	5	43	3.7	1.6	71	14	10	85	36	31	85	48	41	94	40	37
Td	156.2	10.7	91	13	12	23	2	0.5	29	2.5	0.7	43	5.3	2.3	77	17	13.2	85	21	18	94	18	17
Hp	40.5	4.5	82	6.5	5.3	23	3.7	0.9	43	4.3	1.9	43	3.7	1.6	85	9.2	7.8	62	7.8	4.8	89	8.6	7.7
Hs	17.6	2.8	58	2.6	1.5	15	1.5	0.25	14	1	0.14	14	1	0.14	69	2.5	1.8	77	2.2	1.7	50	5.3	2.7
Ap	*	*	85	*	*	61	*	*	0.14	*	*	0	*	*	46	*	*	31	*	*	56	*	*
Pd	*	*	79	*	*	61	*	*	0	*	*	0.14	*	*	69	*	*	38	*	*	78	*	*
M	*	*	61	*	*	54	*	*	0	*	*	0	*	*	8	*	*	0	*	*	28	*	*
Cp	*	*	0	*	*	0	*	*	0	*	*	0.14	*	*	8	*	*	0	*	*	22	*	*
	*	*																					

P = prevalence (%) = no. infected / no.examined
I = mean intensity = total helminths / no. hosts infected
A - abundance = total helminths / no.hosts examined
* - data not available as only presence/absence recorded, not counts

Key to parasites: Ih = *Ixodes holocyclus*; Itr = *I. trichosuri;* Ita = *I. tasmani*
Ap = *Acanthopsylla pavida*; Arr = *A. rothschildi rothschildi*; Co = *Choristopsylla ochi*; Bi = *Bibikovana iridis*; Sd = *Stephanocercus dasyuri*
Tc = *Trichosurolaelaps trichosuri*; Td = *T. dixous*; Hp = *Haemolaelaps penelope*; Hs = *H. sisyphus*; Ap = *Atellana papilio*; Pd = *Petrogalochirus dycei*; M = *Murichirus anabiotus*; Cp = *Cheyletiella parasitivorax*

Table 7.4: Prevalence of the sheathed microfilariae of the filarioid nematode *S. venacavincola* and oocysts of an undescribed *Eimeria* sp. from 104 *T. caninus* from seven study sites. Microfilariae were detected by a filter technique using blood stored in EDTA. Oocysts were detected by faecal flotation.

Site	Prevalence (%)	
	S. venacavincola	*Eimeria* sp.
Cambarville (Vic)	0 (0/32)	25 (8/32)
Bellbird (Vic)	0 (0/13)	31 (4/13)
Barrington Tops (NSW)	22 (5/19)	16 (3/19)
Whian Whian State Forest (NSW)	0 (0/7)	0 (0/7)
Byrangery Reserve (NSW)	57 (4/7)	43 (3/7)
Conondale Ranges (Qld)	31 (4/13)	46 (6/13)
Bulburin State Forest (Qld)	0 (0/13)	31 (4/13)
TOTAL	12 (13/104)	27 (28/104)

Table 7.5: Regression coefficients and standard errors of significant explanatory variables in a statistical model using linear regression of the first principal coordinate in a study of the distribution of helminth species in *T. caninus* in eastern Australia (see also Figure 7.4 and 7.5).

Variable	Regression coefficient	SE
Constant	0.3332	0.0592
Victoria	0*	-
C NSW	-0.559	0.107
N NSW	-0.1478	0.0993
Qld	-0.7879	0.0865

State labels: Victoria = Cambarville and Bellbird
C NSW = Barrington Tops
N NSW = Whian Whian and Byrangery Reserve
Qld = Conondale Ranges and Bulburin State Forest

* The estimate of the regression coefficient for the first level of the categorical variable "State" was set to zero because of constraints due to degrees of freedom.

Table 7.6: Regression coefficients and standard errors of significant explanatory variables in a statistical model using linear regression of the second principal coordinate in a study of the distribution of helminth species in *T. caninus* in eastern Australia (see also Fig 7.4 and 7.5).

Variable	Regression coefficient	SE
Constant	0.0894	0.0610
Victoria	0*	-
C NSW	0.088	0.110
N NSW	-0.422	0.102
Qld	-0.0713	0.0889

State labels: Victoria = Cambarville and Bellbird
C NSW = Barrington Tops
N NSW = Whian Whian and Byrangery Reserve
Qld = Conondale Ranges and Bulburin State Forest

* The estimate of the regression coefficient for the first level of the categorical variable "State" was set to zero because of constraints due to degrees of freedom.

Table 7.7: Regression coefficients and standard errors in a statistical model derived from logistic regression analysis of significant explanatory variables which affect the presence or absence of *Ixodes holocyclus* on *T. caninus*. The variable "Site" which has seven levels, and "Sex" were the only significant explanatory variables

Variable	**Regression coefficient**	**SE**	**p**
Constant	-1.361	0.645	
Site			0.003
Cambarville	0*	-	
Bellbird	0	-	
Whian Whian	3.30	1.24	
Byrangery	2.96	1.17	
Conondales	0.25	1.03	
Bulburin	0.545	0.938	
Barrington Tops	0	-	
Sex	-1.929	0.941	0.02

* The estimate of the regression coefficient for the first level of the categorical variable "Site" was set to zero because of constraints due to degrees of freedom.

Table 7.8: Regression coefficients and standard errors in a statistical model from logistic regression analysis of significant explanatory variables which affect the presence or absence of *Ixodes tasmani* on *T. caninus*. The variable "Site", which has seven levels was the only signficant explanatory variable (p=0.017)

Variable	Regression coefficient	SE
Constant	-2.303	0.606
Cambarville	0*	-
Bellbird	0.799	0.988
Whian Whian	3.22	1.03
Byrangery	2.015	0.975
Conondales	1.492	0.852
Bulburin	0	-
Barrington Tops	1.347	0.801

* The estimate of the regression coefficient for the first level of the categorical variable "Site" was set to zero because of constraints due to degrees of freedom.

Table 7.9: Regression coefficients and standard errors of significant explanatory variables in a statistical model using linear regression of the first principal coordinate in a study of the distribution of species of mites in *T. caninus* in eastern Australia (see also Figs 7.7 and 7.8).

Variable	**Regression coefficient**	**SE**	**p**
Constant	-0.3489	0.0653	
Sex	0.2252	0.0671	<0.001
Group			<0.001
Cambarville	0*	-	
Bellbird males	0.213	0.132	
Bellbird females	0.802	0.148	
Whian Whian	0.757	0.126	
Byrangery	0.708	0.127	
Conondales	0.2099	0.099	
Bulburin	0.366	0.101	
Barrington Tops	0.1677	0.0917	

* The estimate of the regression coefficient for the first level of the categorical variable "group" was set to zero because of constraints due to degrees of freedom.

Table 7.10: Regression coefficients and standard errors of significant explanatory variables in a statistical model using linear regression of the second principal coordinate in a study of the distribution of species of mites in *T. caninus* in eastern Australia (see also Fig 7.7).

Variable	Regression coefficient	SE	p
Constant	0.0833	0.0372	
Site			<0.001
Cambarville	0*	-	
Bellbird males	0.3446	0.0890	
Bellbird females	0.089	0.103	
Whian Whian	-0.1073	0.089	
Byrangery	-0.1534	0.089	
Conondales	-0.2325	0.07	
Bulburin	-03173	0.07	
Barrington Tops	-0.1372	0.0626	

* The estimate of the regression coefficient for the first level of the categorical variable "group" was set to zero because of constraints due to degrees of freedom.

Table 7.11: Blood variables from *T. caninus* for which there was a significant effect of the presence or absence of parasitic infection.

Blood variable	Explanatory variables	p value
log(WCC)	State	0.001
	I. holocyclus	0.001
total protein	State	0.001
	I. trichosuri	0.03
urea	State	0.001
	T. crassipes	0.04
globulins	State	0.001
	I. holocyclus	0.001

Table 7.12: Blood variables from *T. caninus* for which there was a significant effect of the intensity of parasitic infection.

Blood variable	Explanatory variables	p value
log(WCC)	State	0.001
	log(*I. holocyclus*)	0.001
total protein	State	0.001
	log(*I. trichosuri*)	0.02
log(neutrophils)	log(*I. holocyclus*)	0.001

Figure 7.1: Prevalence (%) of helminth species recorded from *T. caninus* across the host geographic range

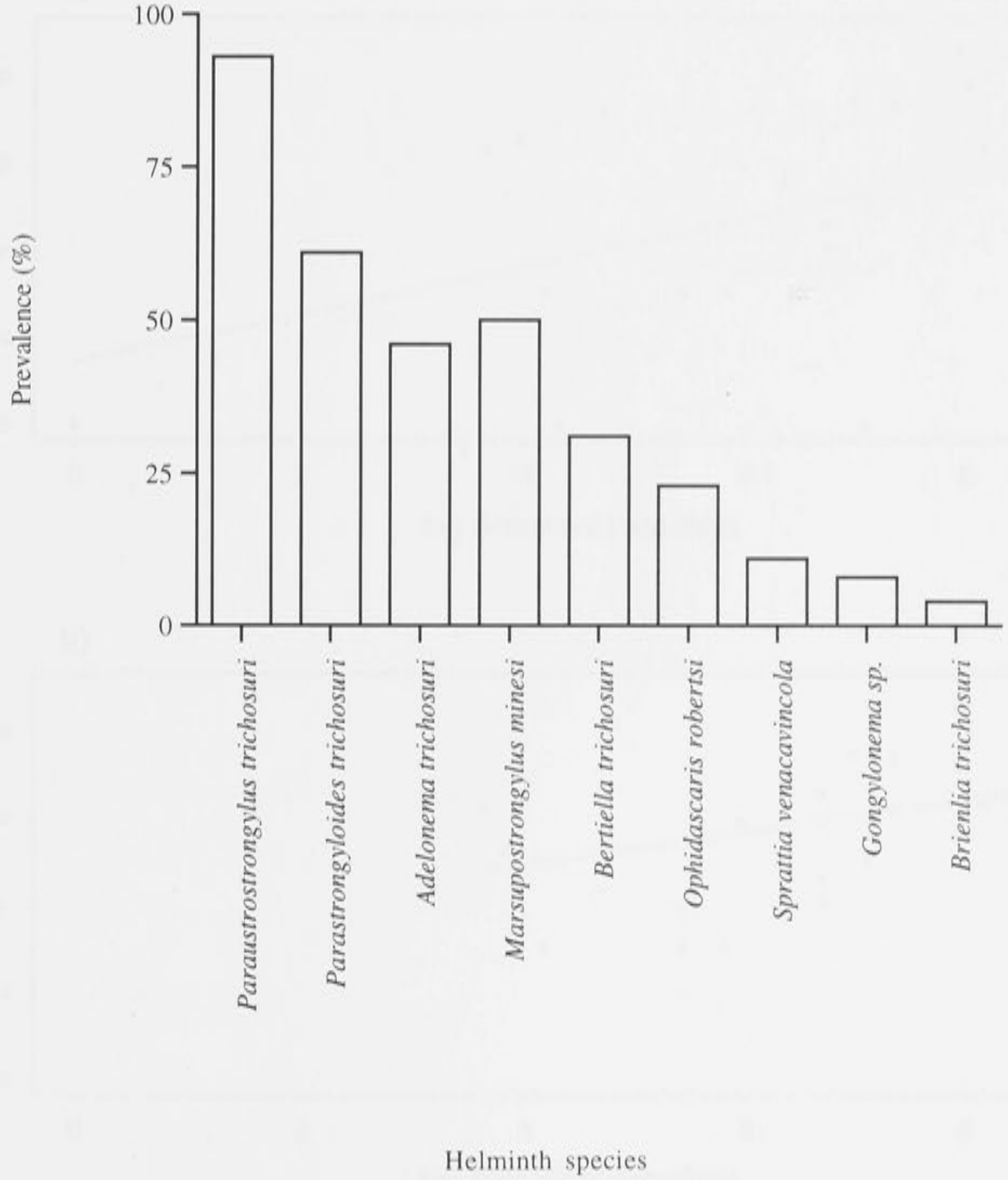


Figure 7.2: Fitted values of the relationship between the faecal egg count and intensity of intestinal parasites of necropsied *T. caninus* using mixed models;
a) zero counts included, **b)** zero counts excluded.

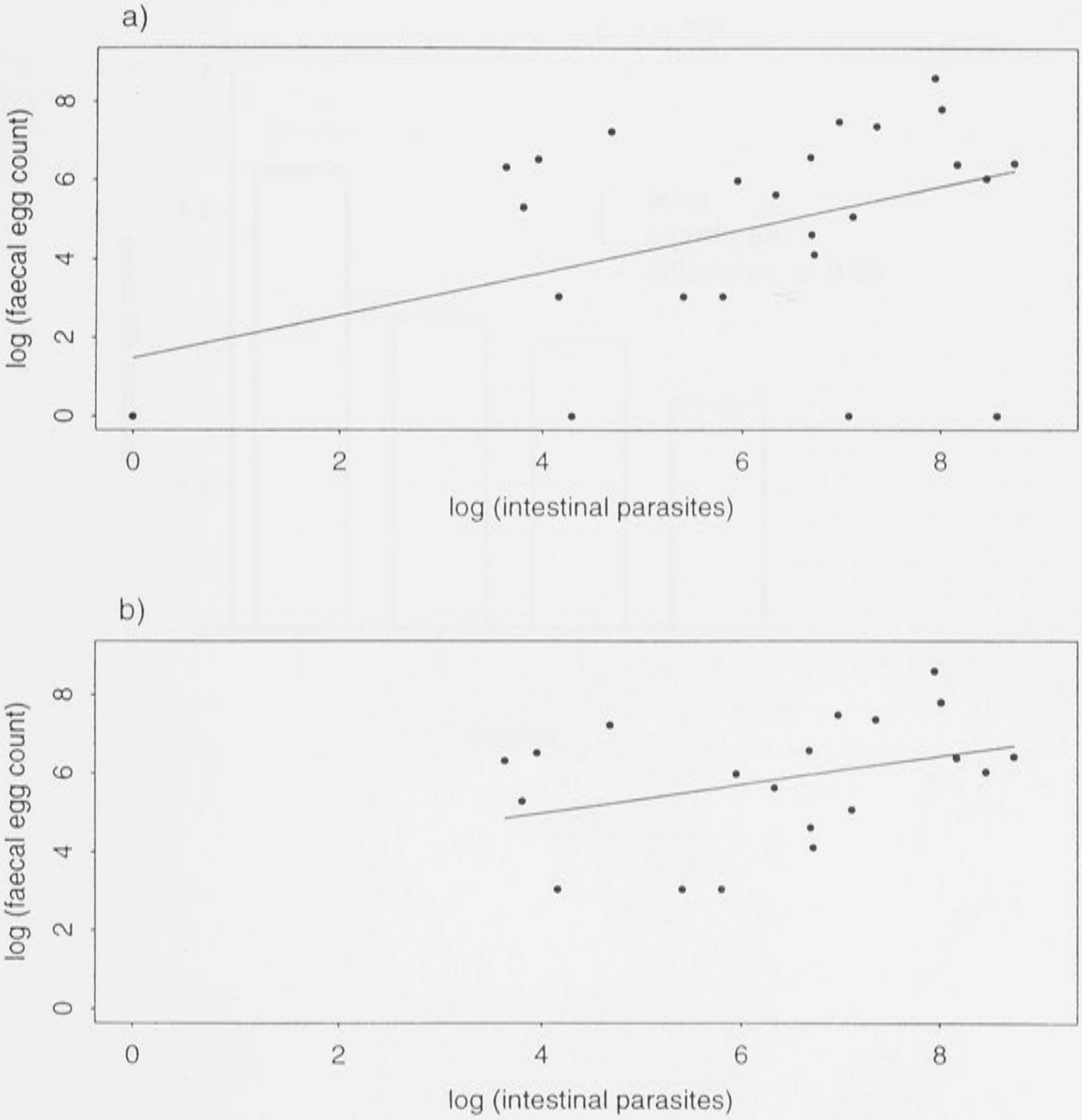


Figure 7.3: Serial egg counts from consecutive faecal samples collected from the distal (1) to proximal (4) rectum and colon of *T. caninus* Host sample sizes for each gut segment were the same (12), so error is shown as least significant difference

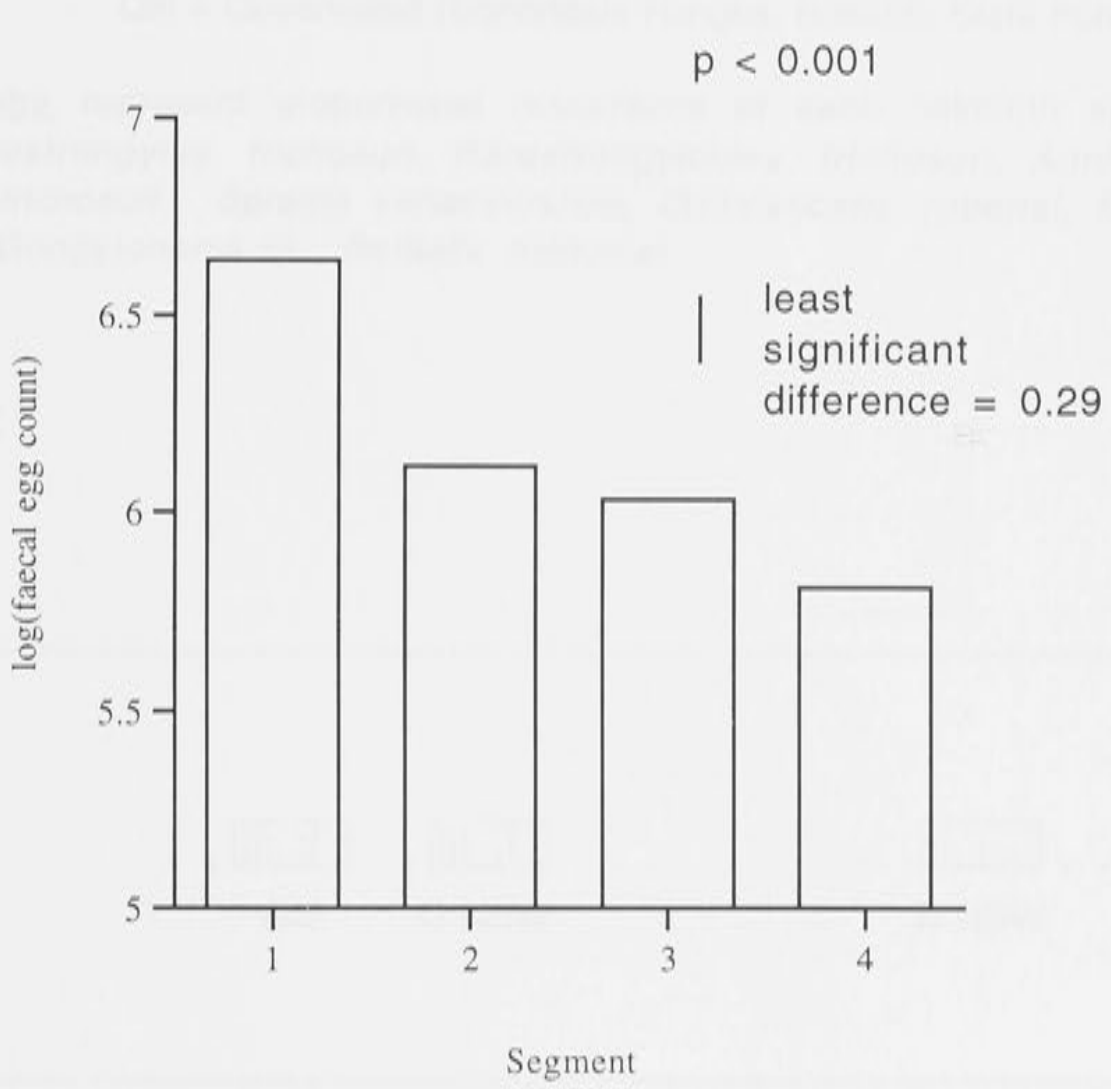


Figure 7.4: Scores of the first principal coordinate from principal coordinate analysis of the occurrence of helminth species recorded from *T. caninus* from four regions within the host geographic range. Patterns of occurrence of helminth species in each region are shown.

Labels: Vic = Victoria (Cambarville and Bellbird);
S NSW = southern New South Wales (Barrington Tops);
N NSW = northern New South Wales (Whian Whian State Forest, Byrangery Reserve, Murwillumbah);
Qld = Queensland (Conondale Ranges, Bulburin State Forest)

Pin graphs represent proportional occurrence of each helminth species as follows: *Paraustrostrongylus trichosuri*, *Parastrongyloides trichosuri*, *Adelonema trichosuri*, *Brienlia trichosuri*, *Sprattia venacavincola*, *Ophidascaris robertsi*, *Marsupostrongylus minesi*, *Gongylonema* sp., *Bertiella trichosuri*.

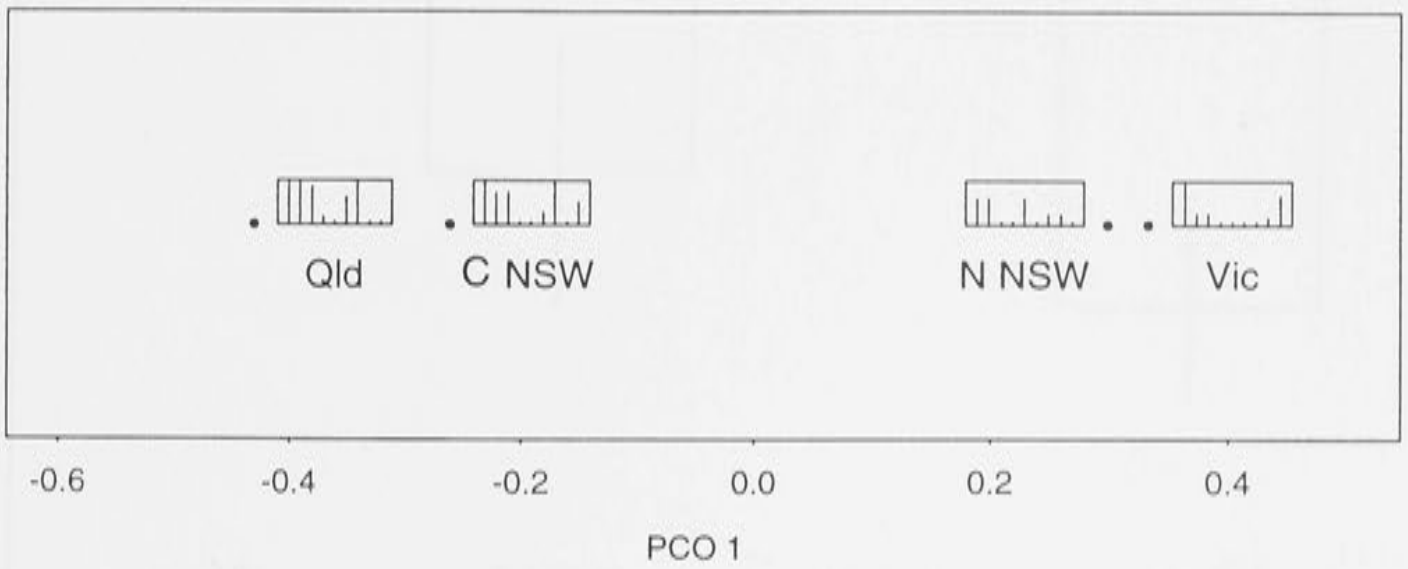


Figure 7.5: Scores from principal coordinate anaysis of helminth species recorded from *T. caninus* from four regions across the host geographic range (means and 95% confidence intervals are shown) (see Table 7.5 and Figure 7.4)

Labels: Vic = Victoria (Cambarville and Bellbird);
S NSW = southern New South Wales (Barrington Tops);
N NSW = northern New South Wales (Whian Whian State Forest, Byrangery Reserve, Murwillumbah);
Qld = Queensland (Conondale Ranges, Bulburin State Forest)

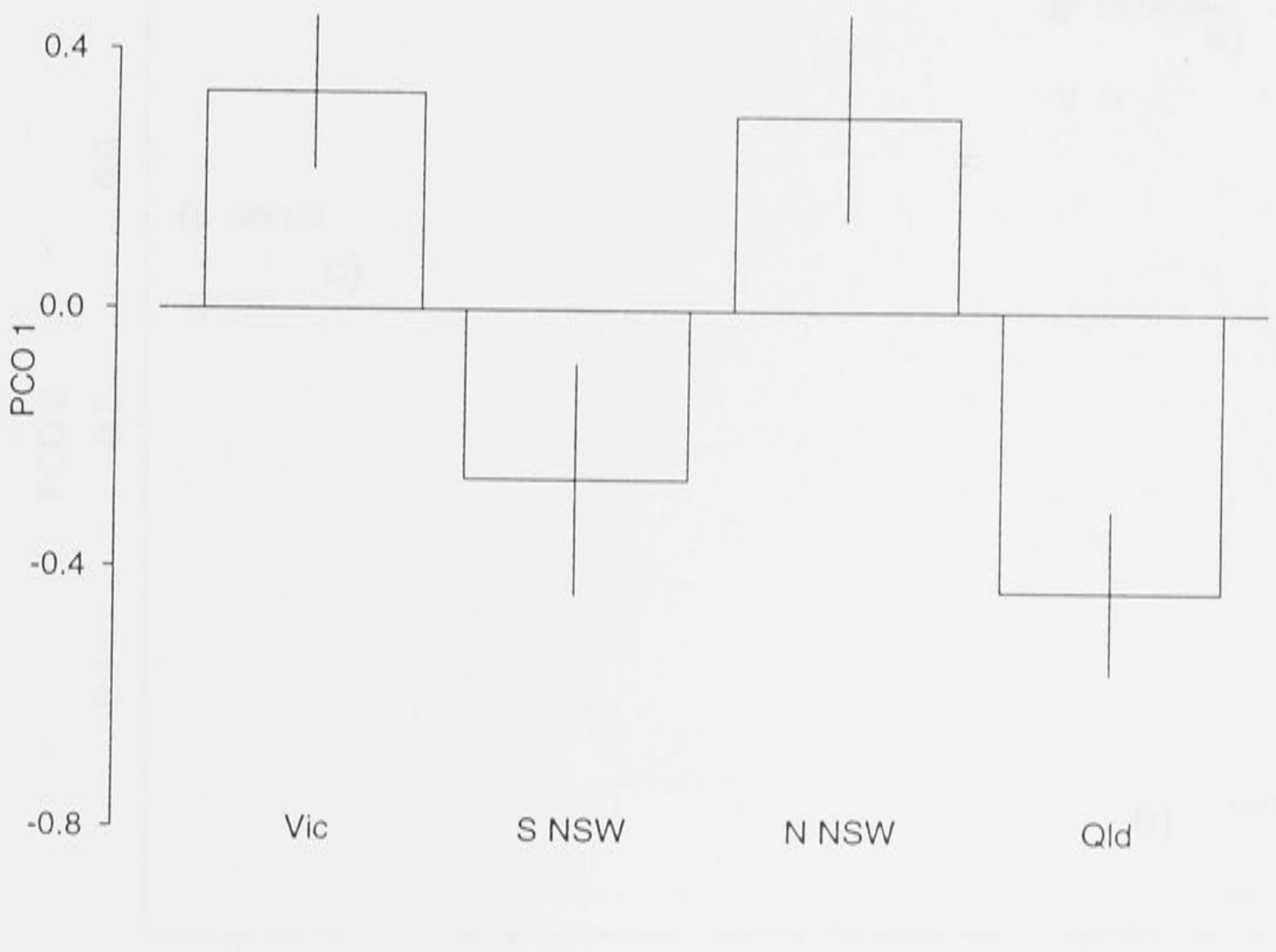


Figure 7.6: Scores of the first two principal coordinates in an analysis of the patterns of occurrence of flea species recorded from *T. caninus*. The number of observations for each state is recorded beside each data point due to overlaying of multiple observations.

Labels: V = Victoria, N = New South Wales, Q = Queensland
a) = *T. caninus* from which no fleas were recorded, or carrying *Stephanocercus dasyuri, Bibikovana iridis* or *Choristopsylla ochi* only
b) = *T. caninus* infested with *Acanthopsylla rothschildi rothschildi* ± other species
c) = *T. caninus* infested with *A. pavida* ± other species

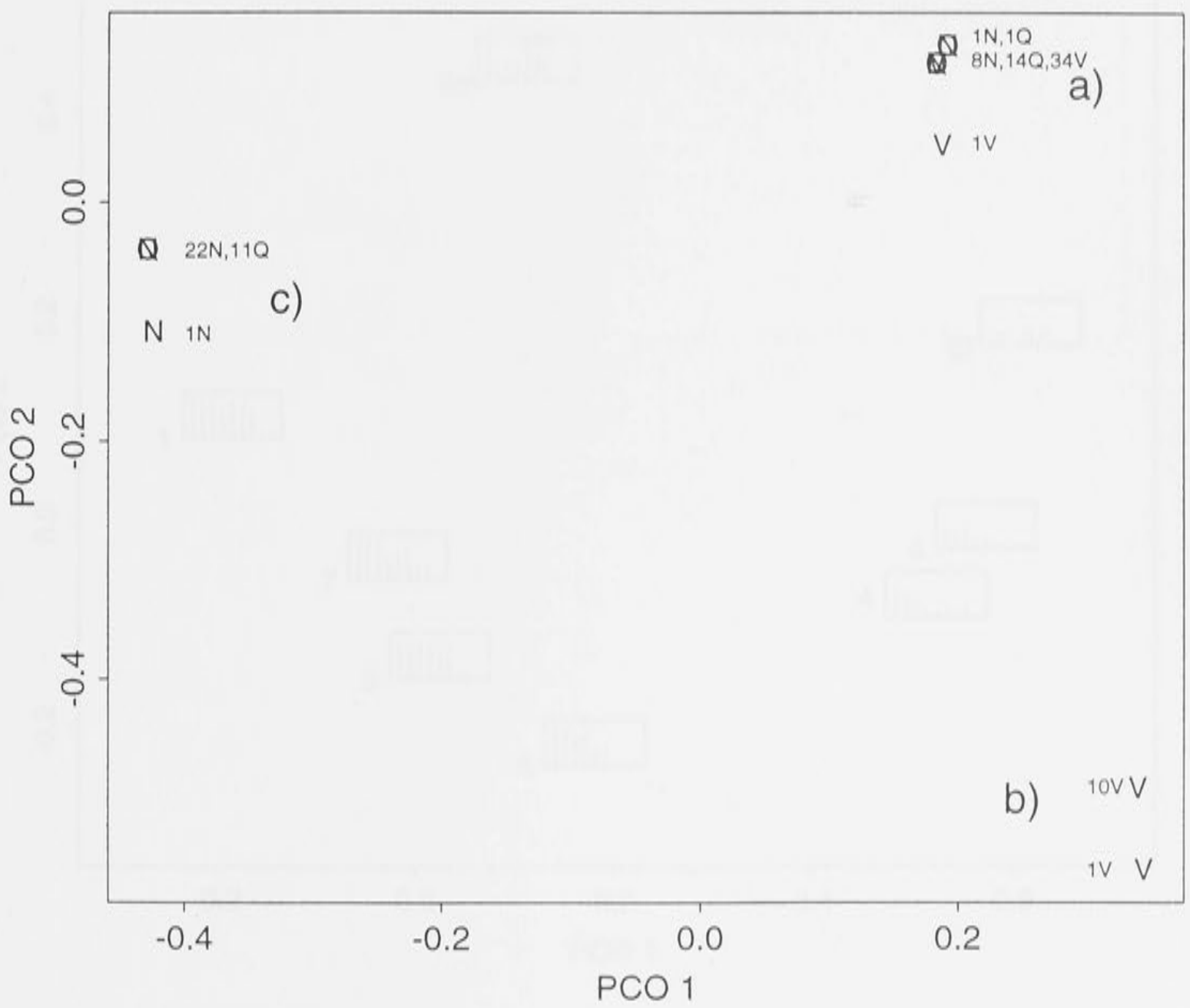


Figure 7.7: Scores of the first two principal coordinates in an analysis of the patterns of occurrence of mites recorded from *T. caninus* from seven study sites; $p<0.001$. Patterns of occurrence of species of mites at each site are shown.

Site labels: 1 = Cambarville, 2m = Bellbird males, 2f = Bellbird females, 3 = Whian Whian State Forest, 4 = Byrangery Reserve, 5 = Conondale Ranges, 6 = Bulburin State Forest, 7 = Barrington Tops.

Pin graphs represent proportional occurrence of each mite species as follows:
T. crassipes, T. dixous, H. penelope, H. sisyphus, A. papilio, P. dycei, M. anabiotus, C. parasitivorax, chiggers.

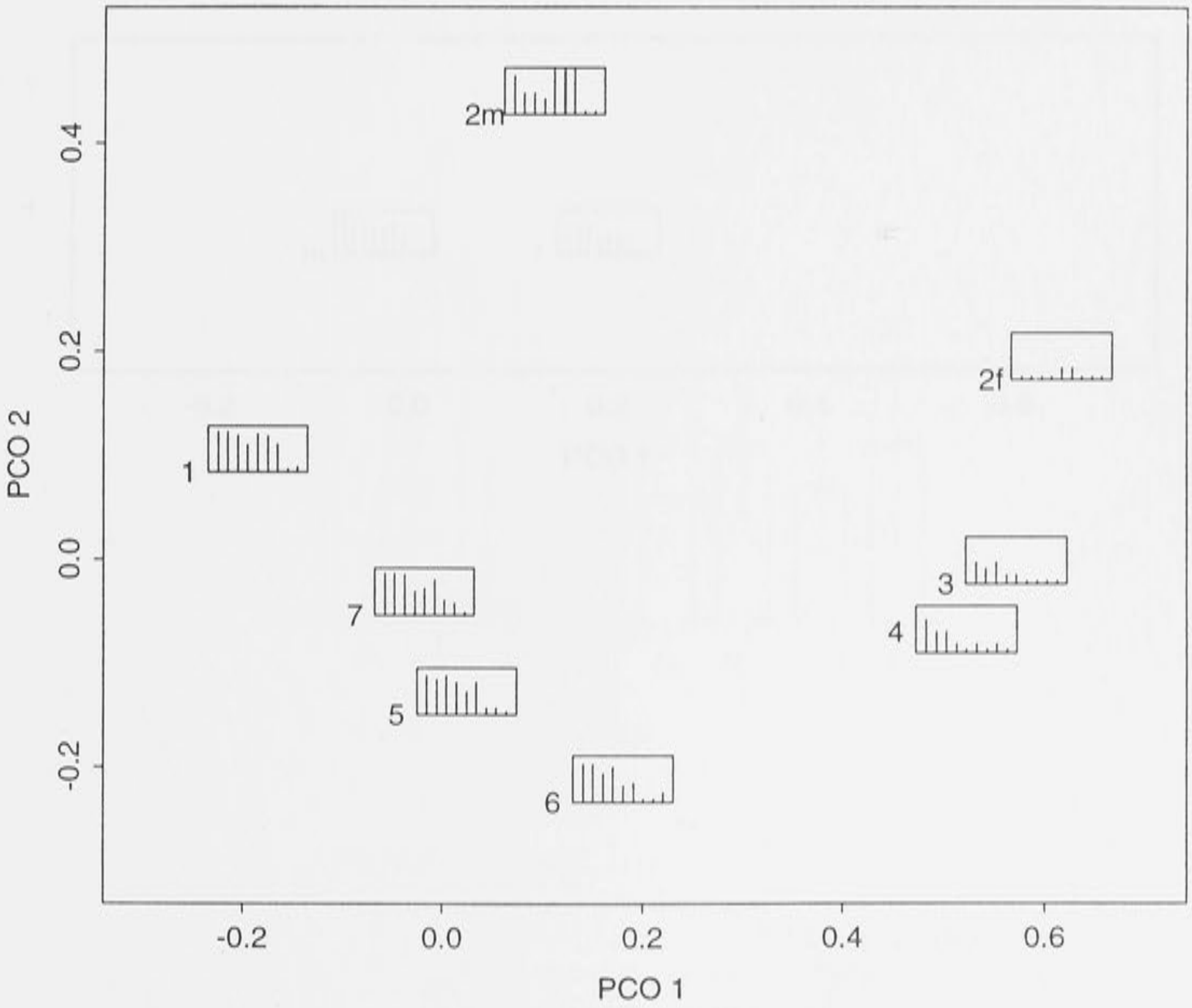


Figure 7.8: Scores of the first principal coordinate in an analysis of the patterns of occurrence of mites recorded from male and female *T. caninus* from seven study sites across the host geographic range; $p<0.001$; m = males, f = females (see Table 7.9).

Pin graphs represent proportional occurrence of each mite species as follows: *T. crassipes, T. dixous, H. penelope, H. sisyphus, A. papilio, P. dycei, M. anabiotus, C. parasitivorax*, chiggers.

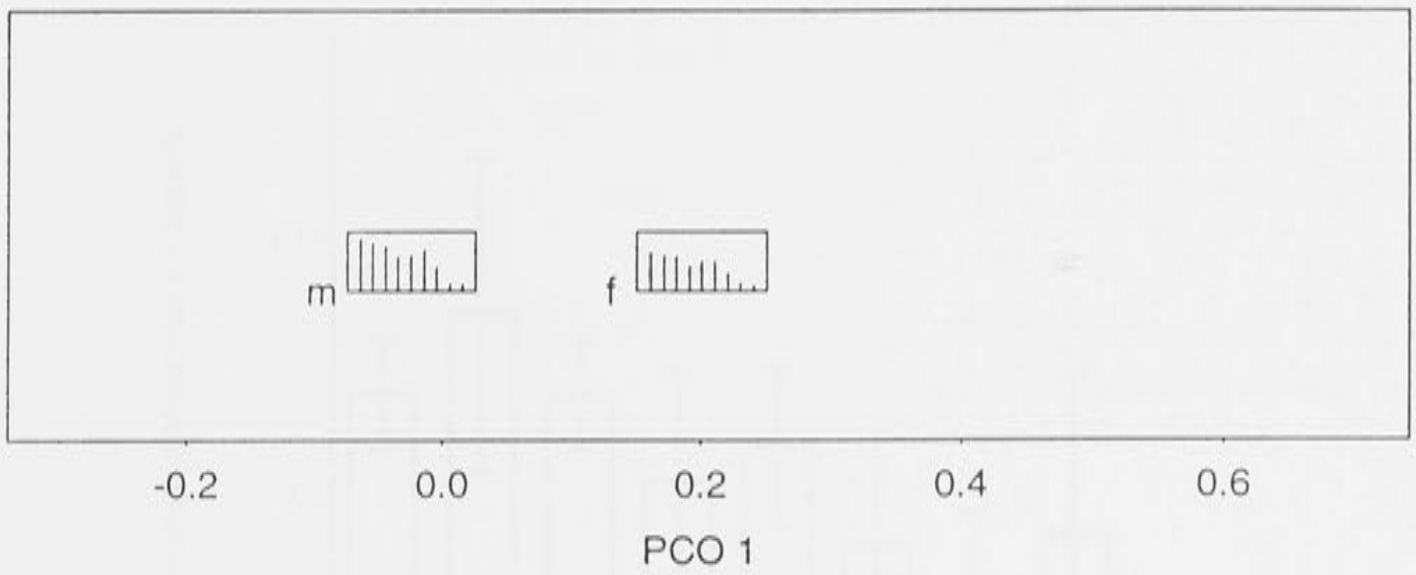
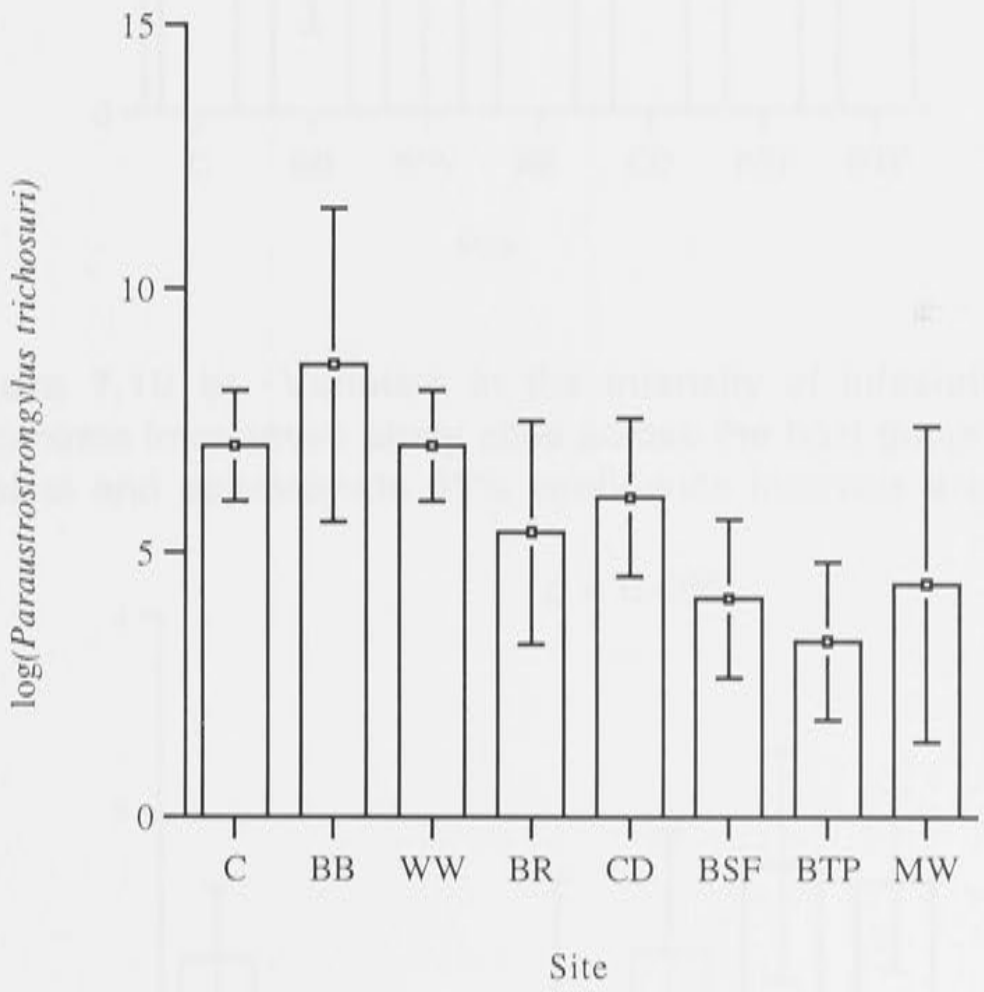


Figure 7.9: Variation in the intensity of infection with *Paraustrostrongylus trichosuri* in *T. caninus* from seven study sites across the host geographic range (approximate 95% confidence intervals are shown)



Site labels: C = Cambarville (Vic)
BB = Bellbird (Vic)
WW = Whian Whian State Forest (NSW)
BR = Byrangery Reserve (NSW)
CD = Conondale Ranges (Qld)
BSF - Bulburin State Forest (Qld)
BTP = Barrington Tops (NSW)
MW = Murwillumbah (NSW)

Figure 7.10 a: Variation in the intensity of infestation with *T. crassipes* of *T. caninus* from seven study sites across the host geographic ranges (means and approximate 95% confidence intervals are shown)

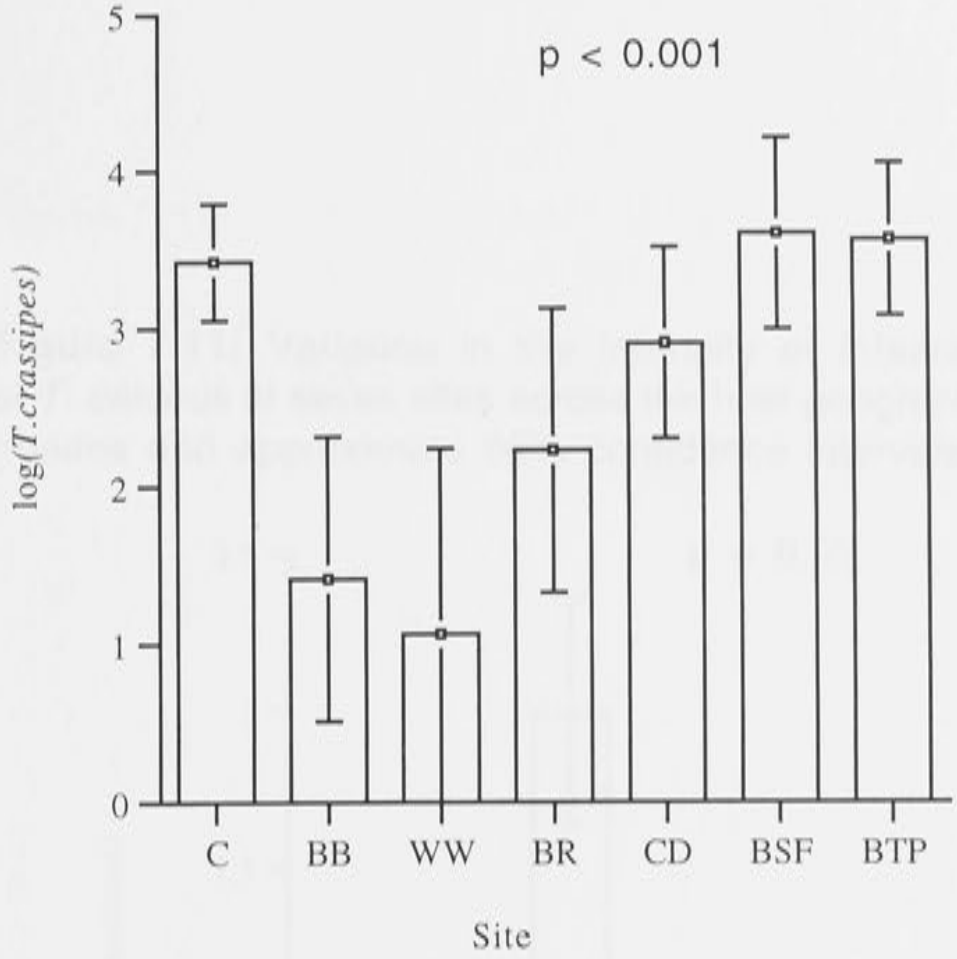
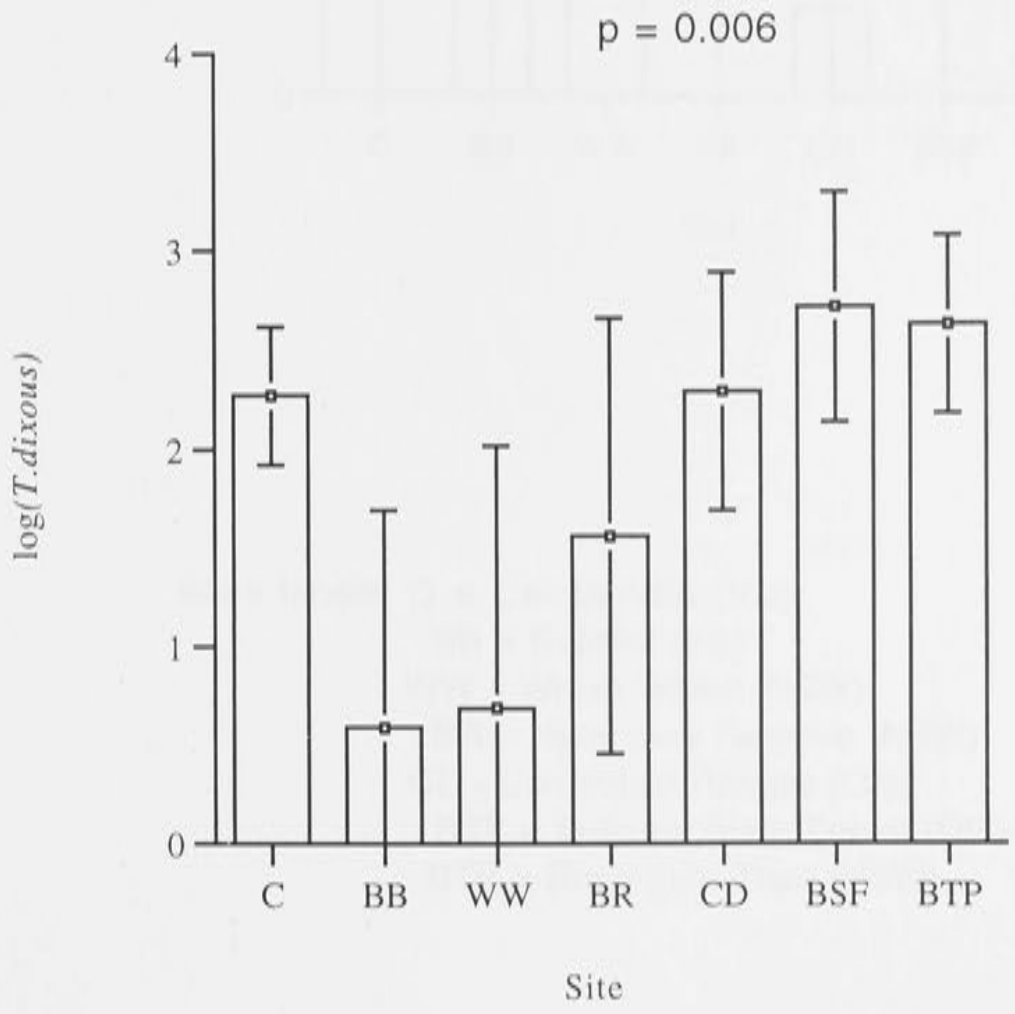
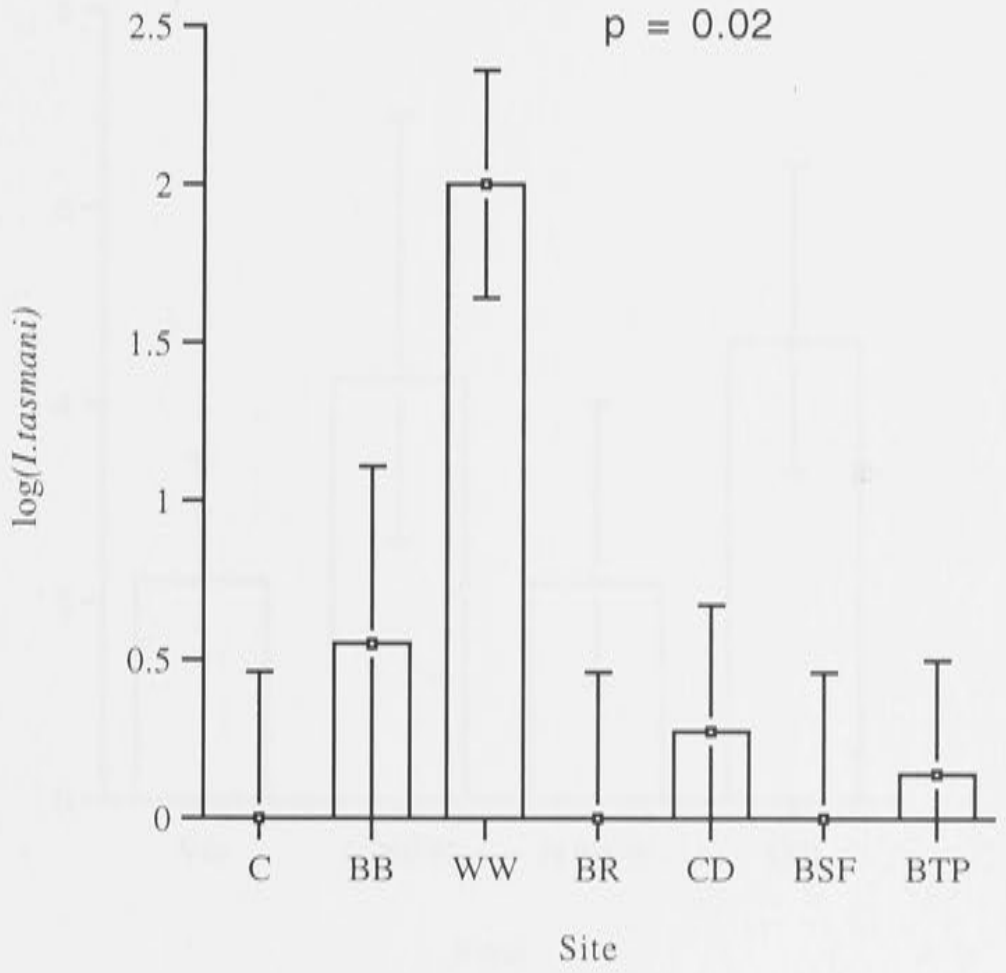


Figure 7.10 b: Variation in the intensity of infestation with *T. dixous* of *T. caninus* from seven study sites across the host geographic range (means and approximate 95% confidence intervals are shown)



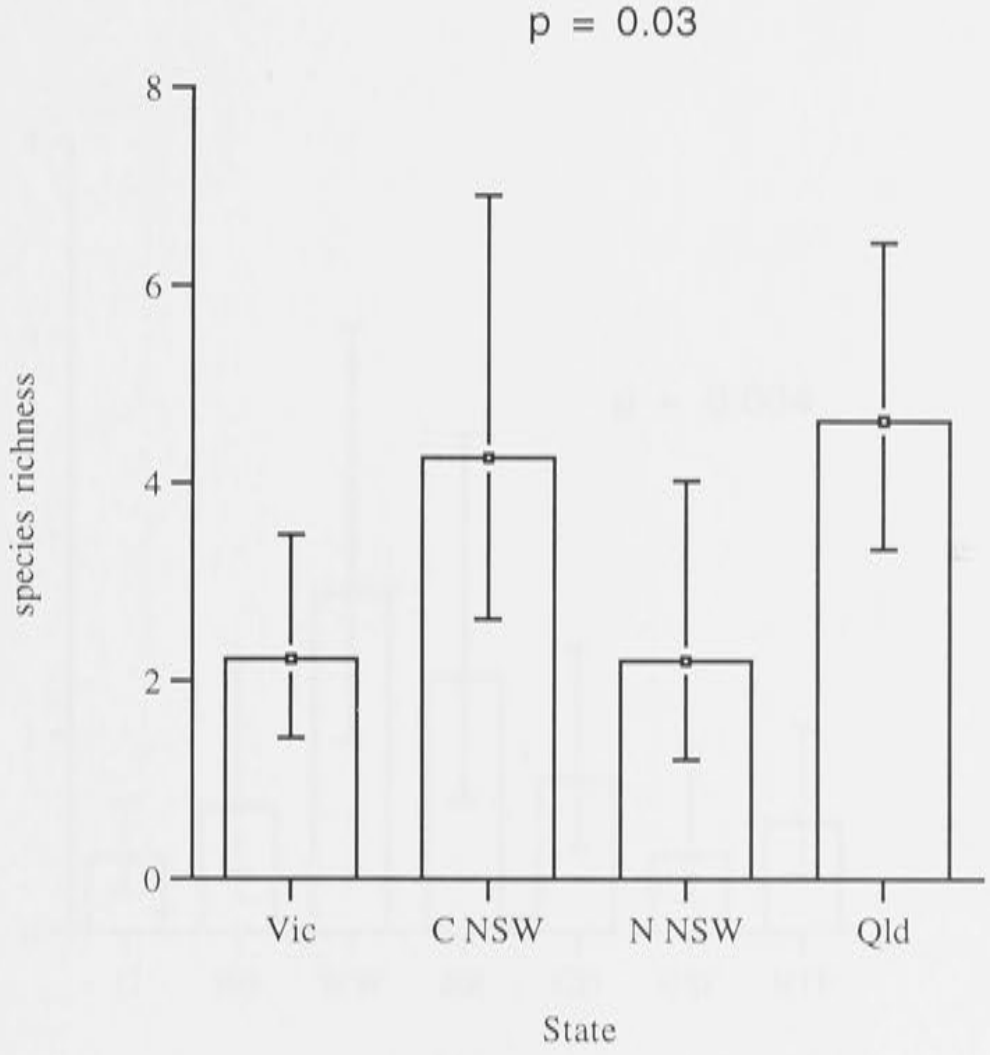
Site labels: C = Cambarville (Vic); BB = Bellbird (Vic); WW = Whian Whian (NSW); BR = Byrangery Reserve (NSW); CD = Conondale Ranges (Qld); BSF = Bulburin State Forest (Qld); BTP = Barrington Tops (NSW)

Figure 7.11: Variation in the intensity of infestation with *I.tasmani* of *T. caninus* at seves sites across the host geographic range (means and approximate 95% confidence intervals are shown)



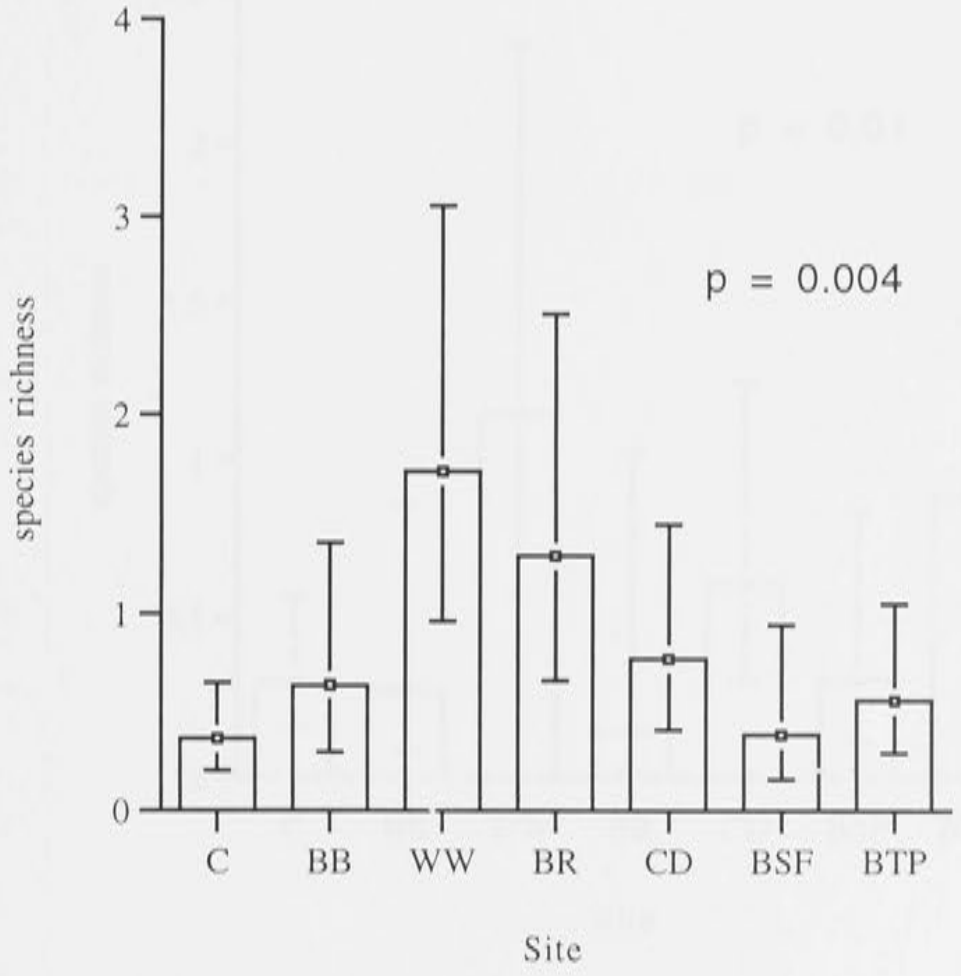
Sites labels: C = Cambarville (Vic)
BB = Bellbird (Vic)
WW = Whian Whian (NSW)
BR = Byrangery Reserve (NSW)
CD - Conondale Ranges (Qld)
BSF = Bulburin State Forest (Qld)
BTP = Barrington Tops (NSW)

Figure 7.12: Species richness of helminth communities recorded from *T. caninus* from four regions within the host geographic range
(means and approximate 95% confidence intervals are shown)



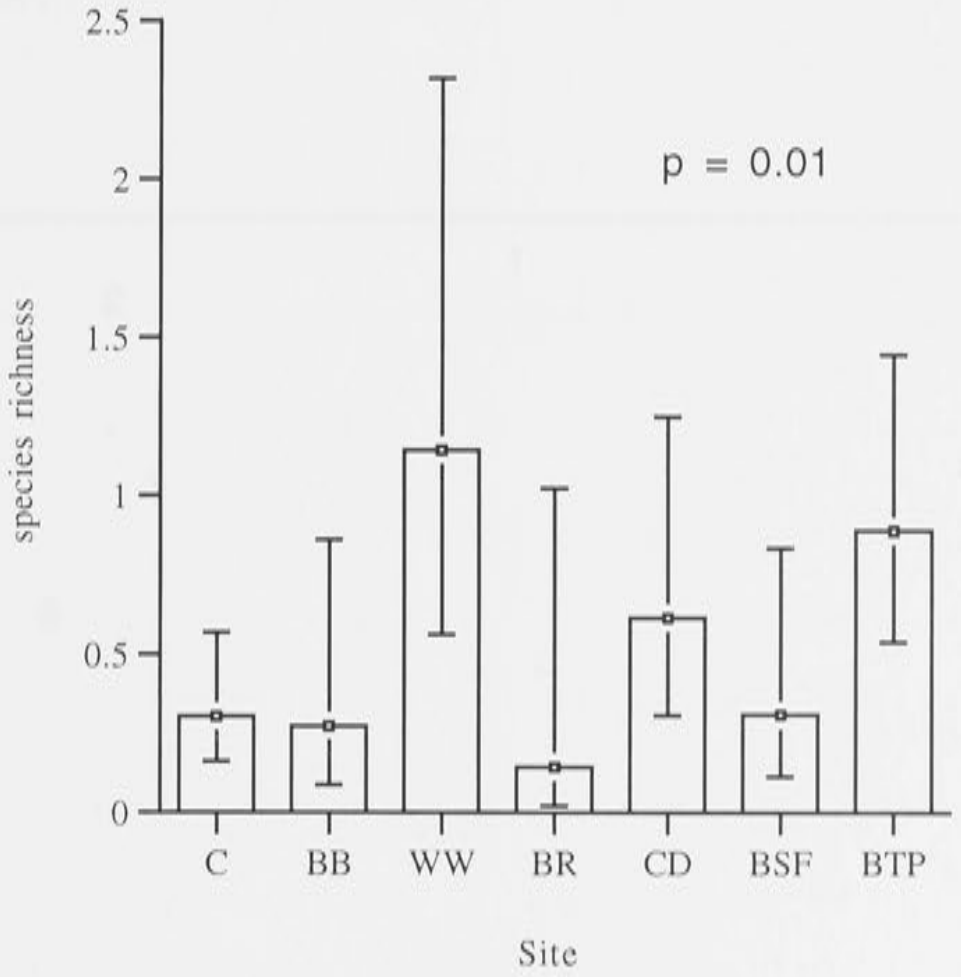
State labels: Vic = Cambarville, Bellbird
C NSW = Barrington Tops
N NSW = Whian Whian, Byrangery
Qld = Conondale Ranges, Bulburin

Figure 7.13: Species richness of ticks recorded from *T. caninus* at seven study sites in eastern Australia (means and approximate 95% confidence intervals are shown)



Key to Sites:
C = Cambarville (Vic)
BB = Bellbird (Vic)
WW = Whian Whian State Forest (NSW)
BR = Byrangery Reserve (BR)
CD = Conondale Ranges (Qld)
BSF = Bulburin State Forest (Qld)
BTP = Barrington Tops (NSW)

Figure 7.14: Species richness of fleas recorded from *T. caninus* at seven study sites in eastern Australia (means and approximate 95% confidence intervals are shown)



Key to Sites:
C = Cambarville (Vic)
BB = Bellbird (Vic)
WW = Whian Whian State Forest (NSW)
BR = Byrangery Reserve (NSW)
CD = Conondale Ranges (Qld)
BSF = Bulburin State Forest (Qld)
BTP = Barrington Tops (NSW)

Figure 7.15: Scores for first two coordinates from principal coordinates analysis of the patterns of co-occurrence of helminth species recorded from *T. caninus* [rare species excluded (prevalence<10%)]. Parasite infracommunities ordinated to examine associations between parasite species.

Labels: 1 = *Paraustrostrongylus trichosuri*, 2 = *Parastrongyloides trichosuri*, 3 = *Adelonema trichosuri*, 4 = *Ophidascaris robertsi*, 5 = *Marsupostrongylus minesi*, 6 = *Bertiella trichosuri*.

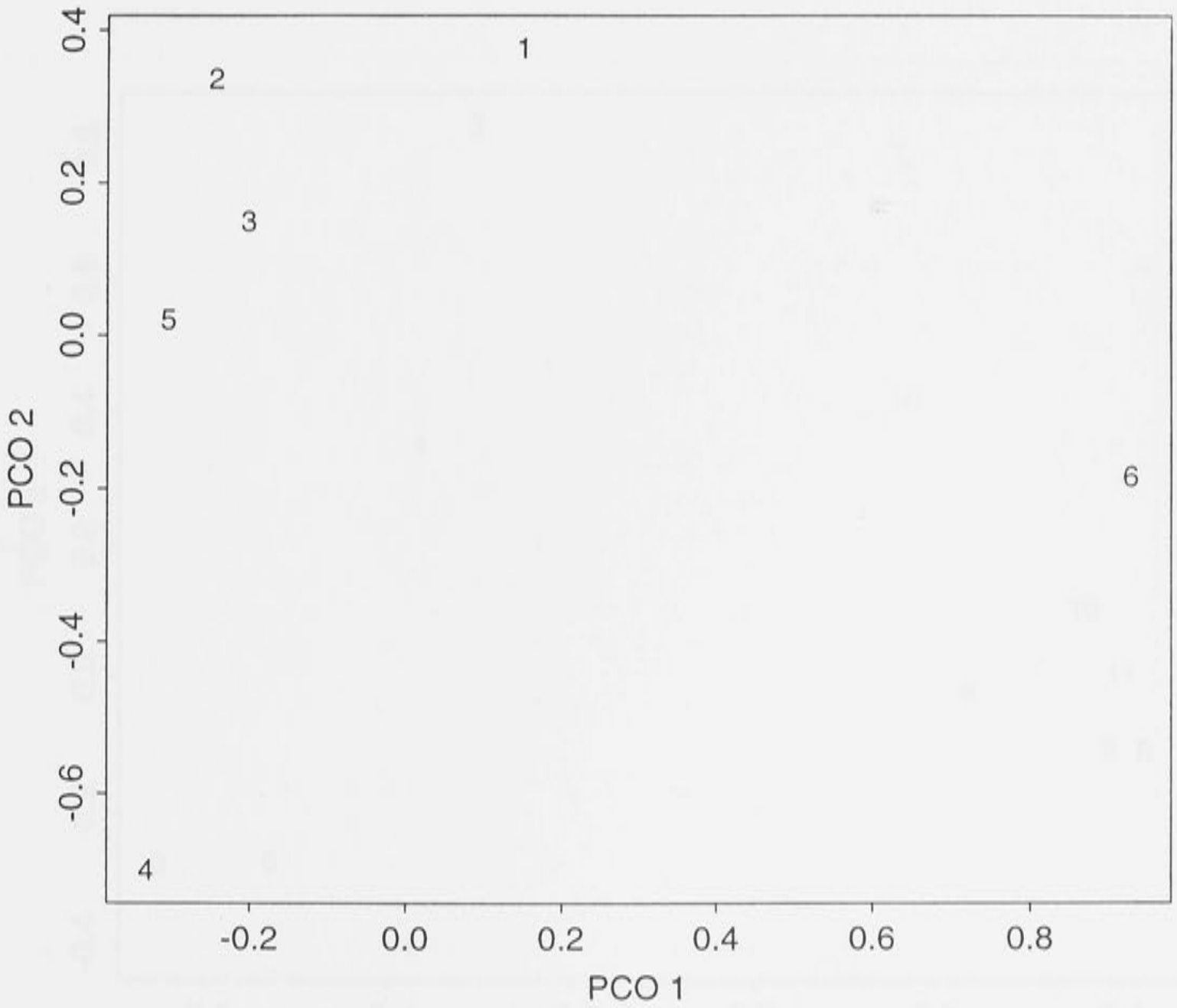


Figure 7.16 Scores for the first two coordinates from principal coordinates analysis of the patterns of co-occurrence of arthropod ectoparasites recorded from *T. caninus* [rare species excluded(prevalence<10%)]. Parasite infracommunities ordinated to examine associations between parasite species.

Labels: 1 = *Acanthopsylla pavida*, 2 = *A. rothschildi rothschildi*, 3 = *Choristopsylla ochi*, 4 = *Ixodes holocyclus*, 5 = *I. trichosuri*, 6 = *I. tasmani*, 7 = *Trichosurolaelaps crassipes*, 8 = *T. dixous*, 9 = *Haemolaelaps penelope*, 10 = *H. sisyphus*, 11 = *Cheyletiella parasitivorax*.

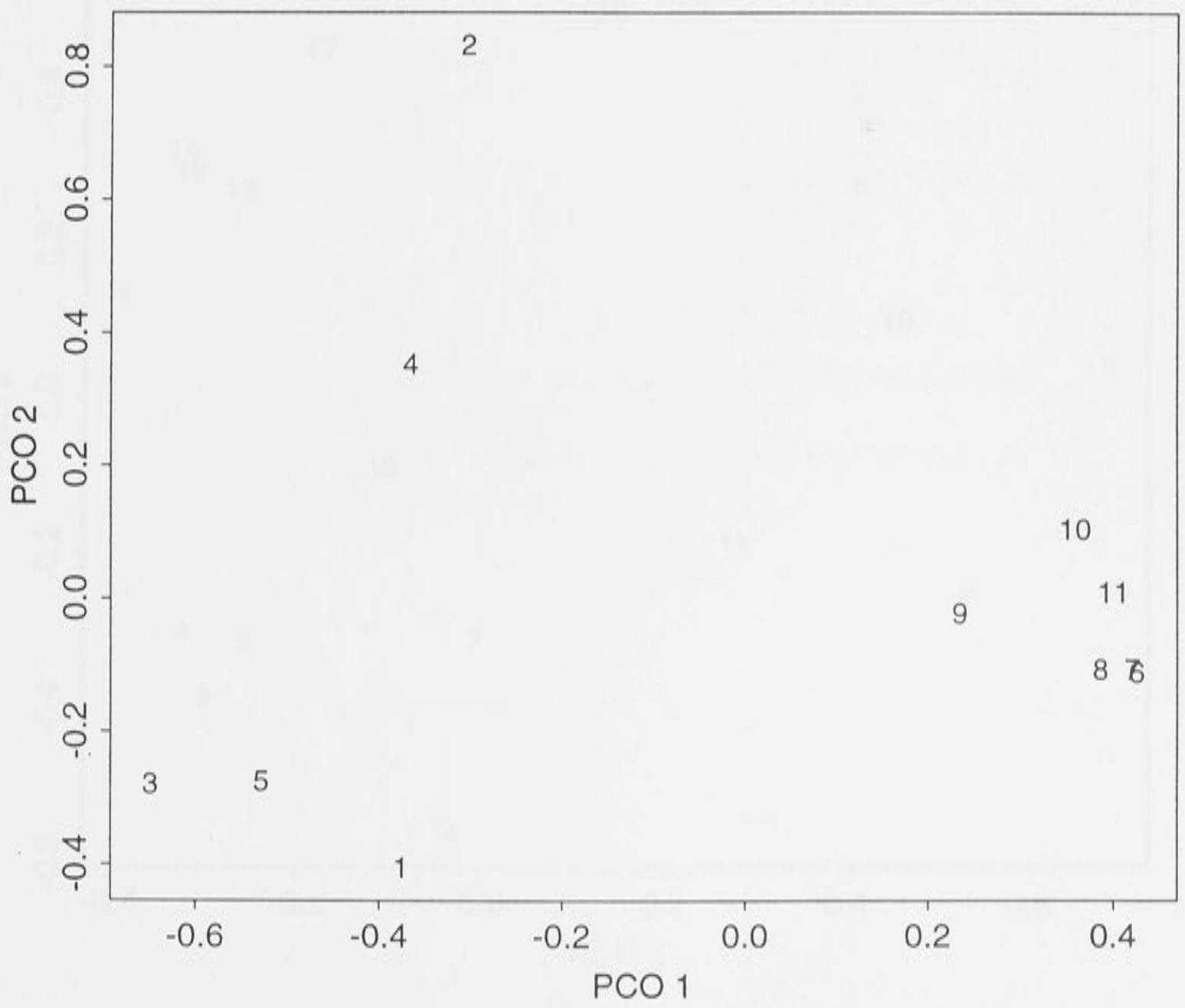


Figure 7.17: Scores for the first two coordinates from principal coordinates analysis of the patterns of co-occurrence of helminth and ectoparasite species recorded from *T. caninus* [rare species excluded (prevalence<10%)]. Parasite infracommunities ordinated to examine associations between parasite species.

Labels: 1 = *Paraustrostrongylus trichosuri*, 2 = *Parastrongyloides trichosuri*, 3 = *Adelonema trichosuri*, 4 = *Ophidascaris robertsi*, 5 = *Marsupostrongylus minesi*, 6 = *Bertiella trichosuri*, 7 = *Acanthopsylla pavida*, 8 = *A. rothschildi rothschildi*, 9 = *Choristopsylla ochi*, 10 = *Ixodes holocyclus*, 11 = *I. trichosuri*, 12 = *I. tasmani*, 13 = *Trichosurolaelaps crassipes*, 14 = *T. dixous*, 15 = *Haemolaelaps penelope*, 16 = *H. sisyphus*, 17 = *Cheyletiella parasitivorax*..

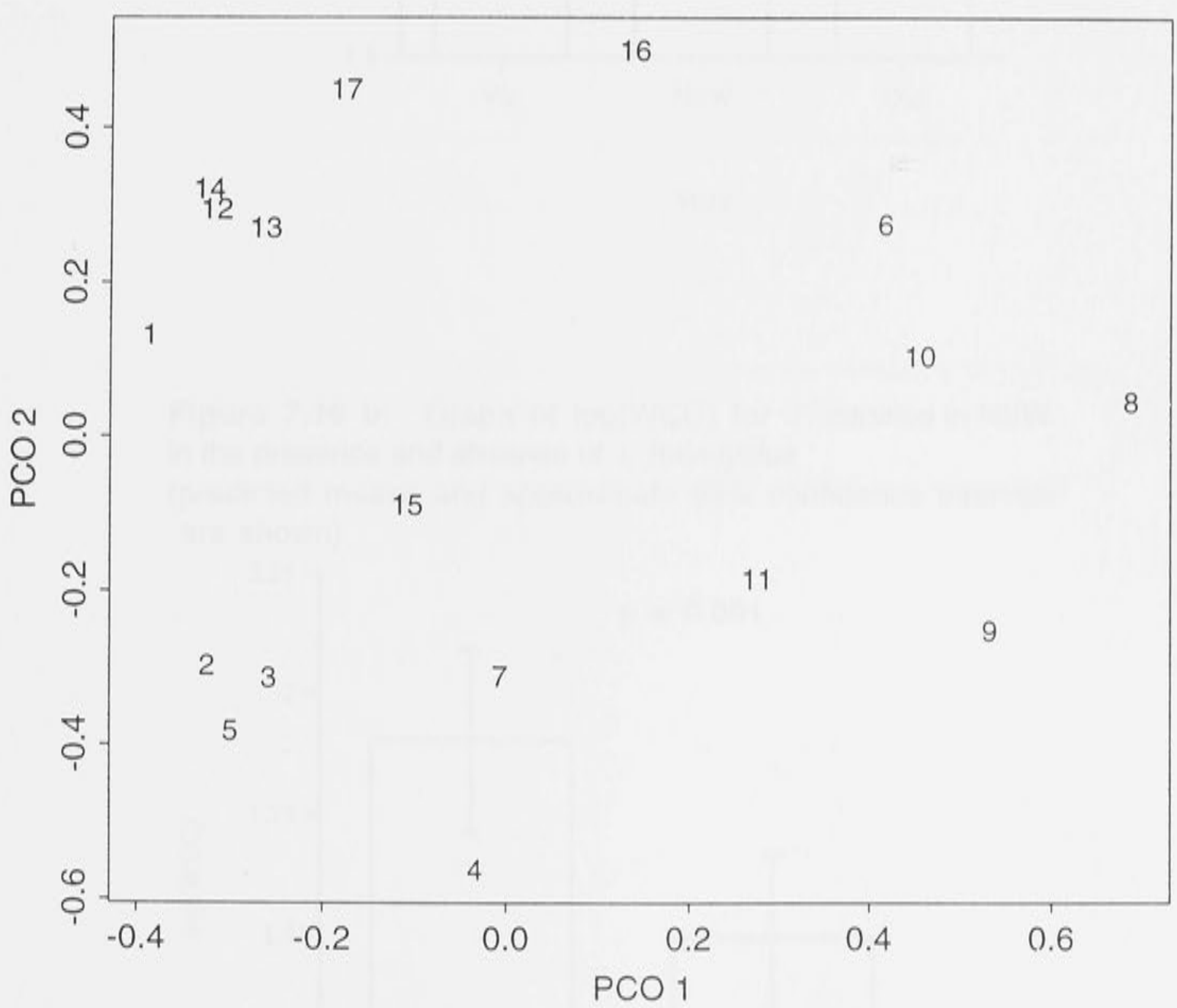


Figure 7.18 a: Graph of log(WCC) for *T. caninus* in Victoria, NSW and Queensland, in the presence of *I. holocyclus*
(predicted means and approximate 95% confidence intervals are shown)

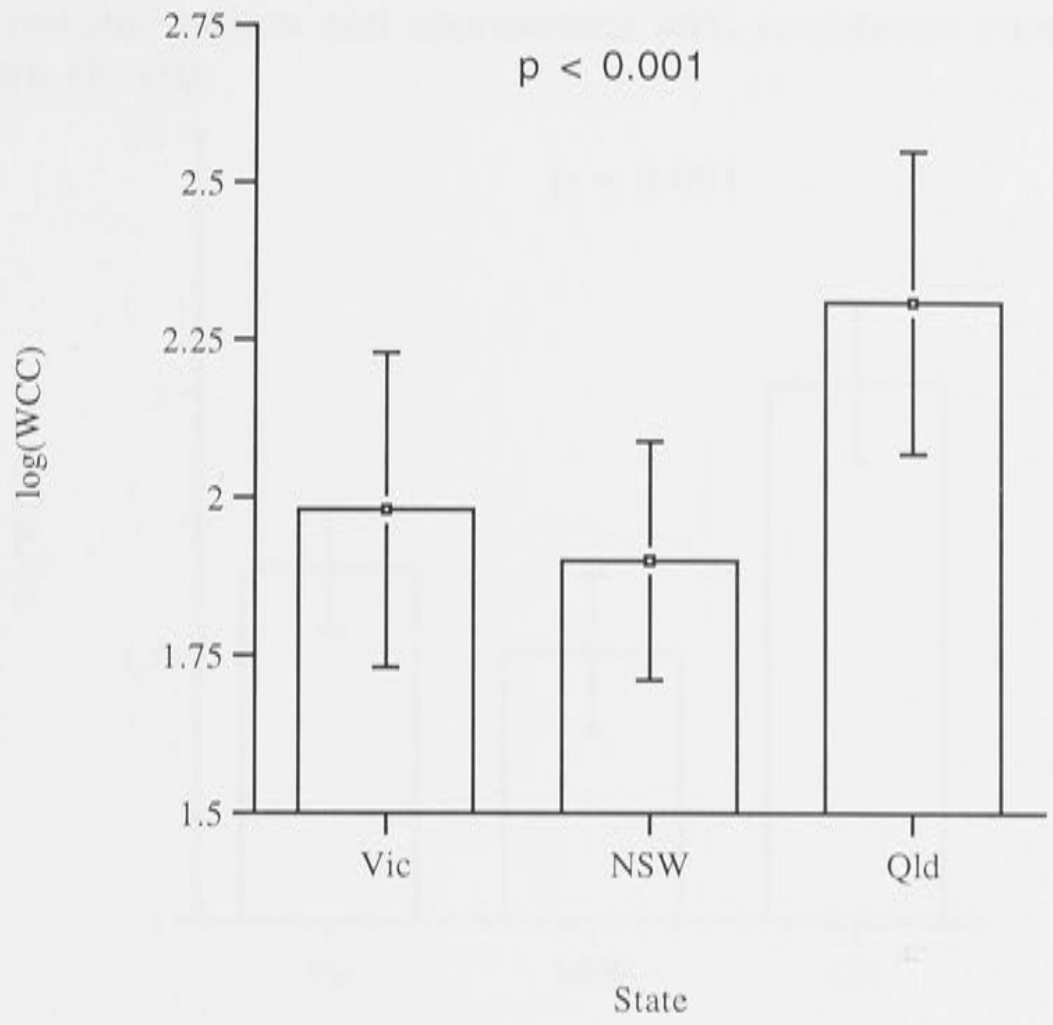


Figure 7.18 b: Graph of log(WCC) for *T. caninus* in NSW in the presence and absence of *I. holocyclus*
(predicted means and approximate 95% confidence intervals are shown)

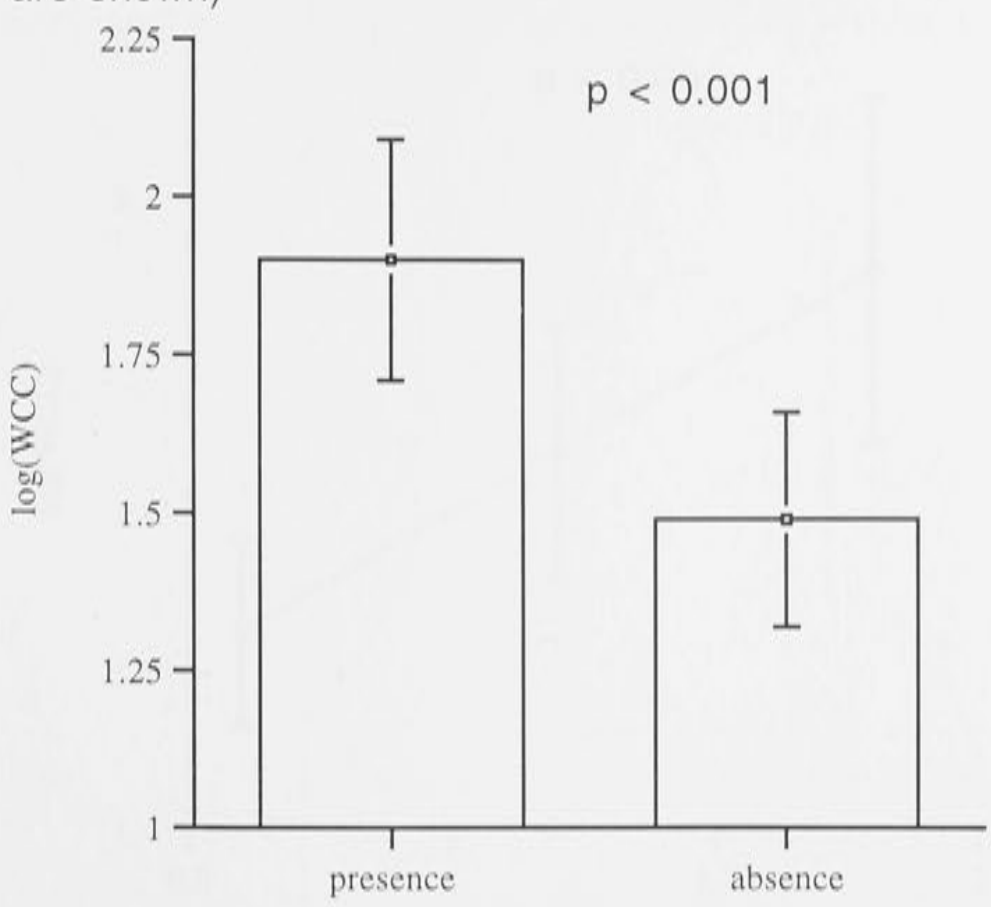


Figure 7.19 a: Graph of log(WCC) for *T. caninus* in three states given a mean intensity of infestation with *I. holocyclus* (predicted means and approximate 95% confidence intervals are shown)

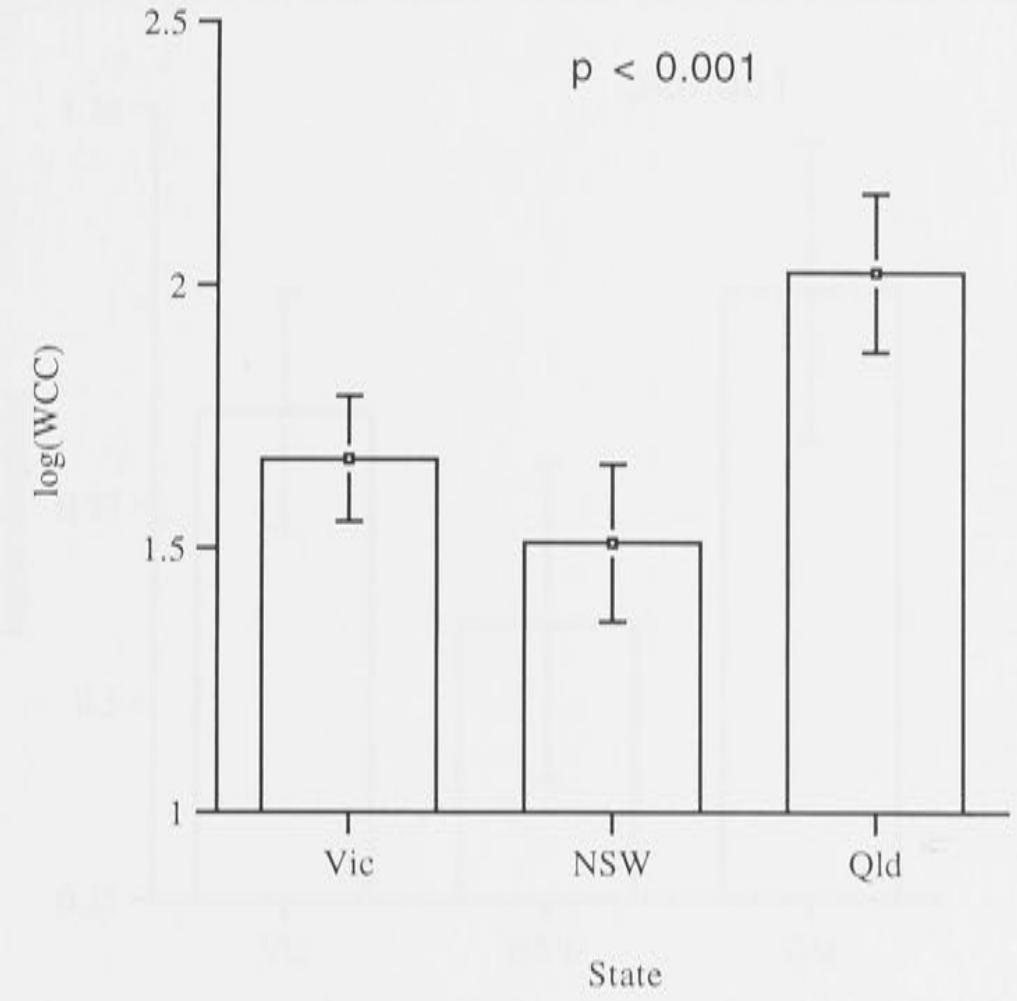


Figure 7.19 b: Graph of the relationship between log(WCC) and log (intensity of infestation with *I. holocyclus)* for *T. caninus* in NSW
(predicted means and approximate 95% confidence intervals are shown)

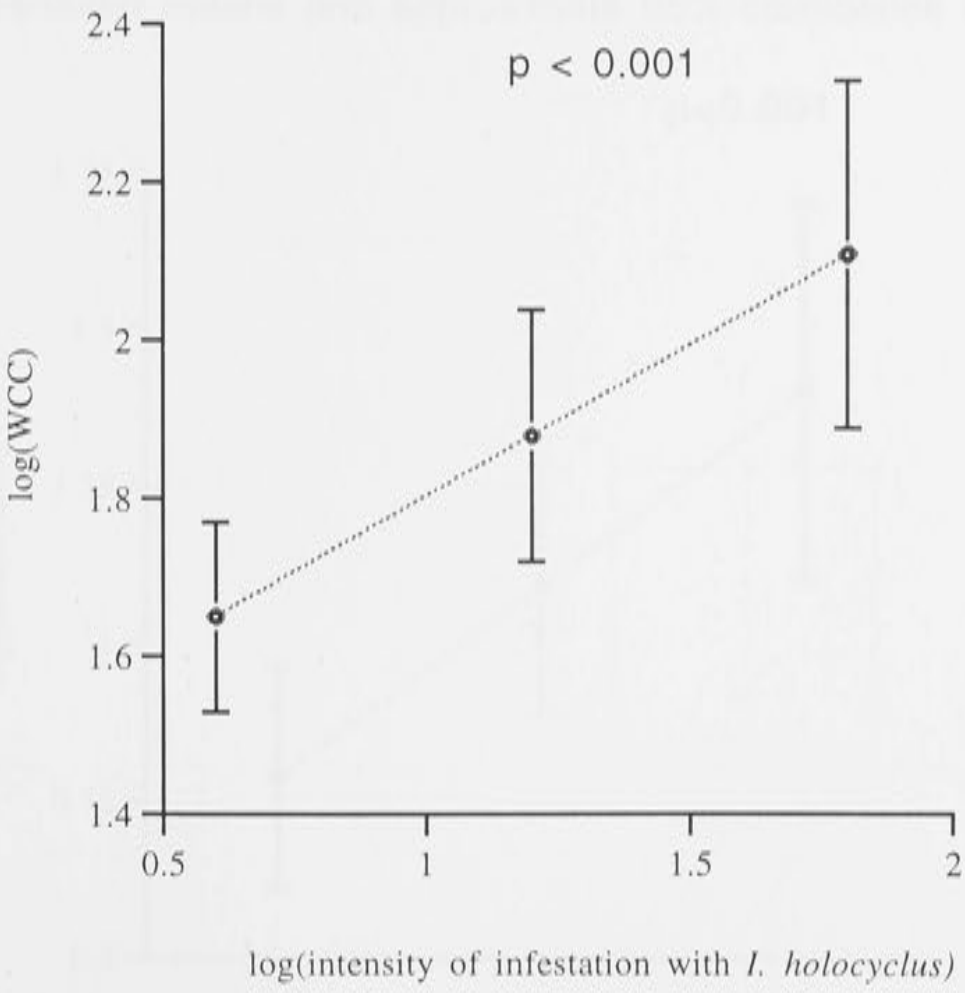


Figure 7.20 a: Graph of log(neutrophils) for *T. caninus* in three states for animals given a mean intensity of infestation with *I. holocyclus* (predicted means and approximate 95% confidence intervals are shown)

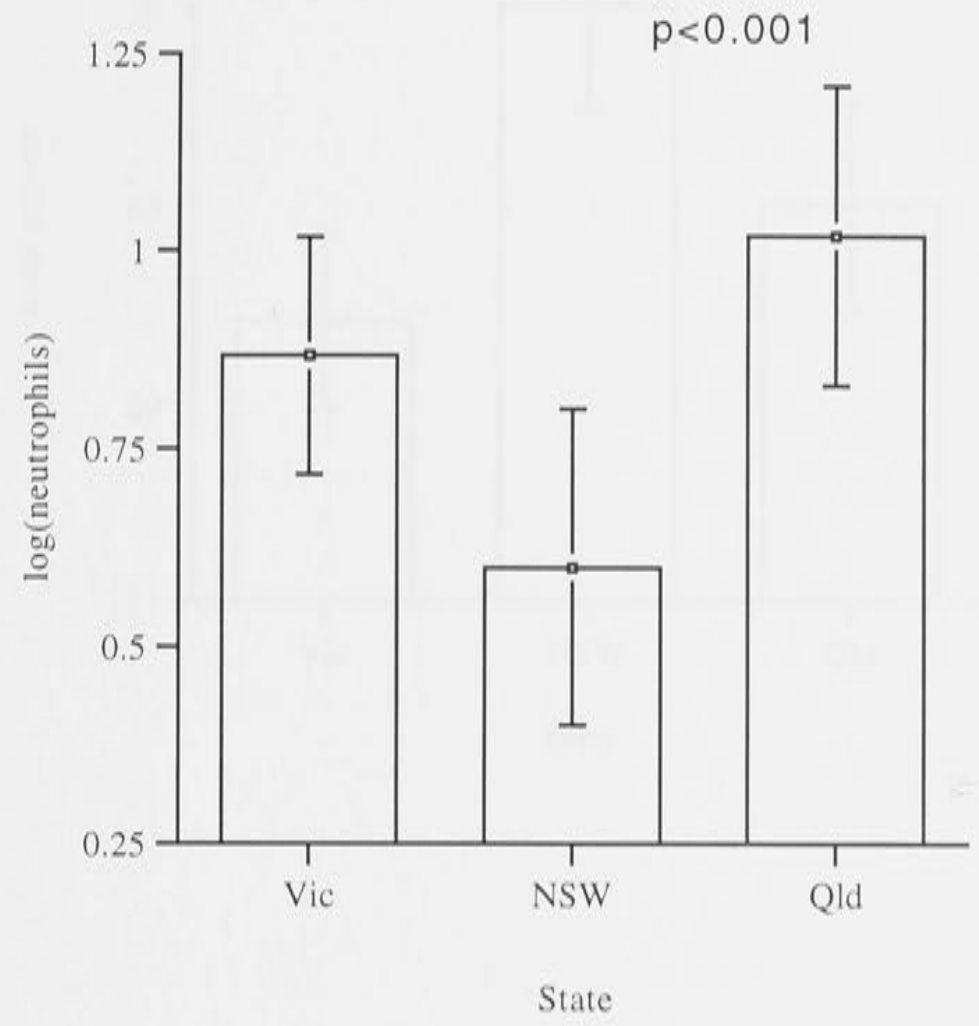


Figure 7.20 b: Graph of the relationship between log(absolute neutrophils) and log(intensity of infestation with *I. holocyclus)* for *T. caninus* in NSW (predicted means and approximate 95% confidence intervals are shown)

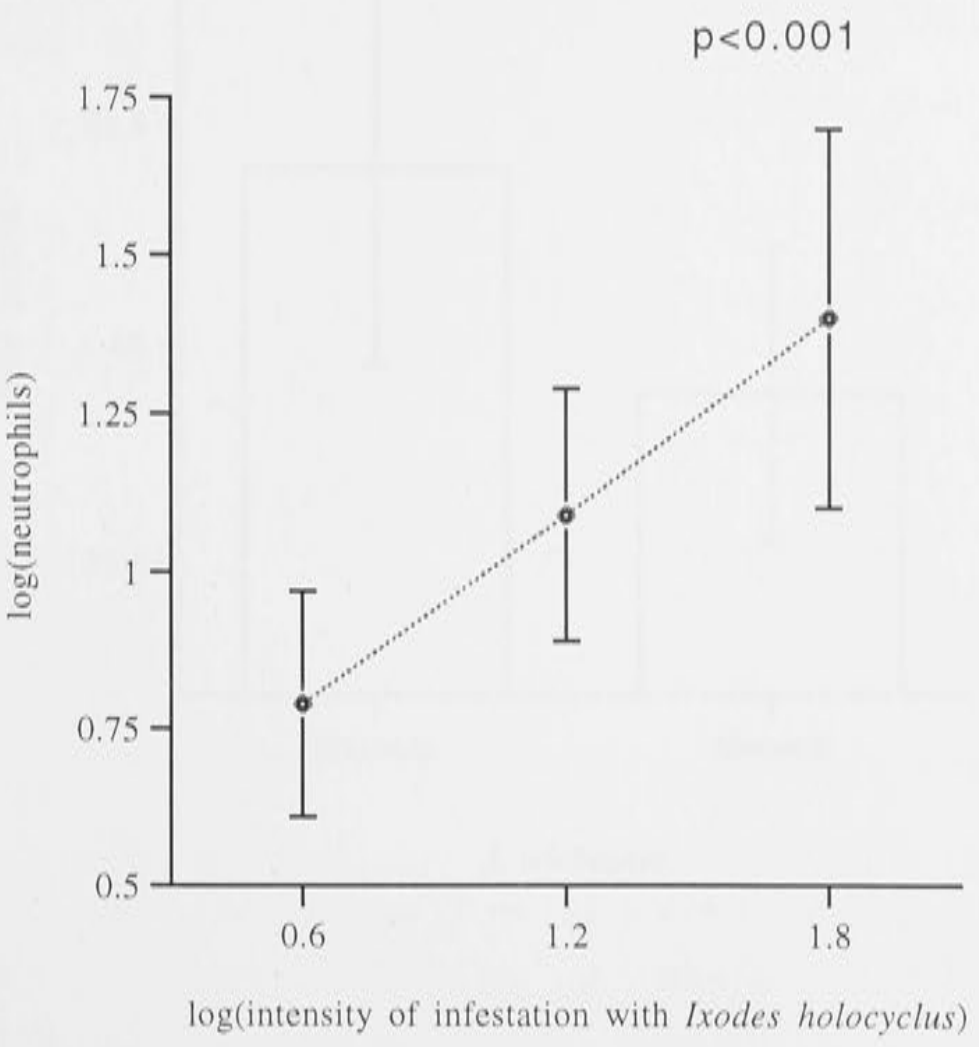


Figure 7.21 a: Graph of total serum protein levels in *T. caninus* in the presence of *I. trichosuri* (predicted means and approximate 95% confidence intervals are shown)

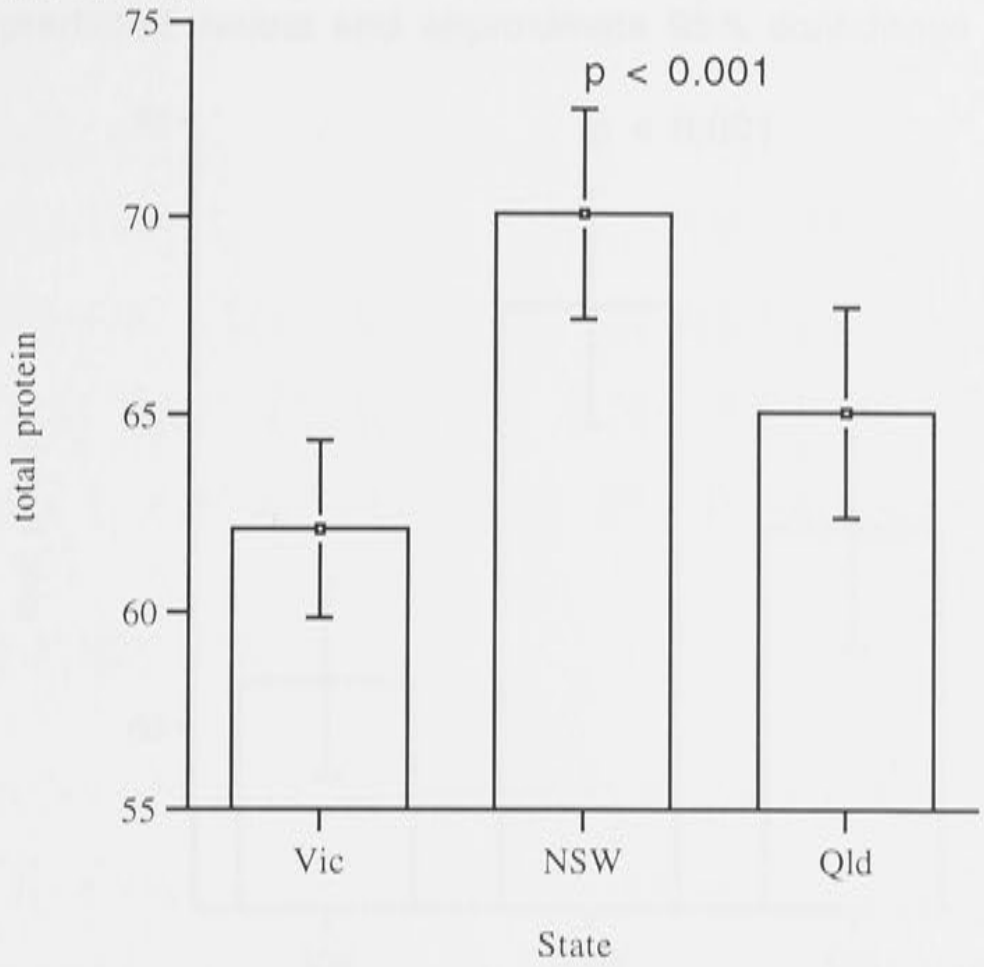


Figure 7.21 b: Graph of total serum protein levels of *T. caninus* in Victoria in the presence and absence of *I. trichosuri* (predicted means and approximate 95% confidence intervals are shown)

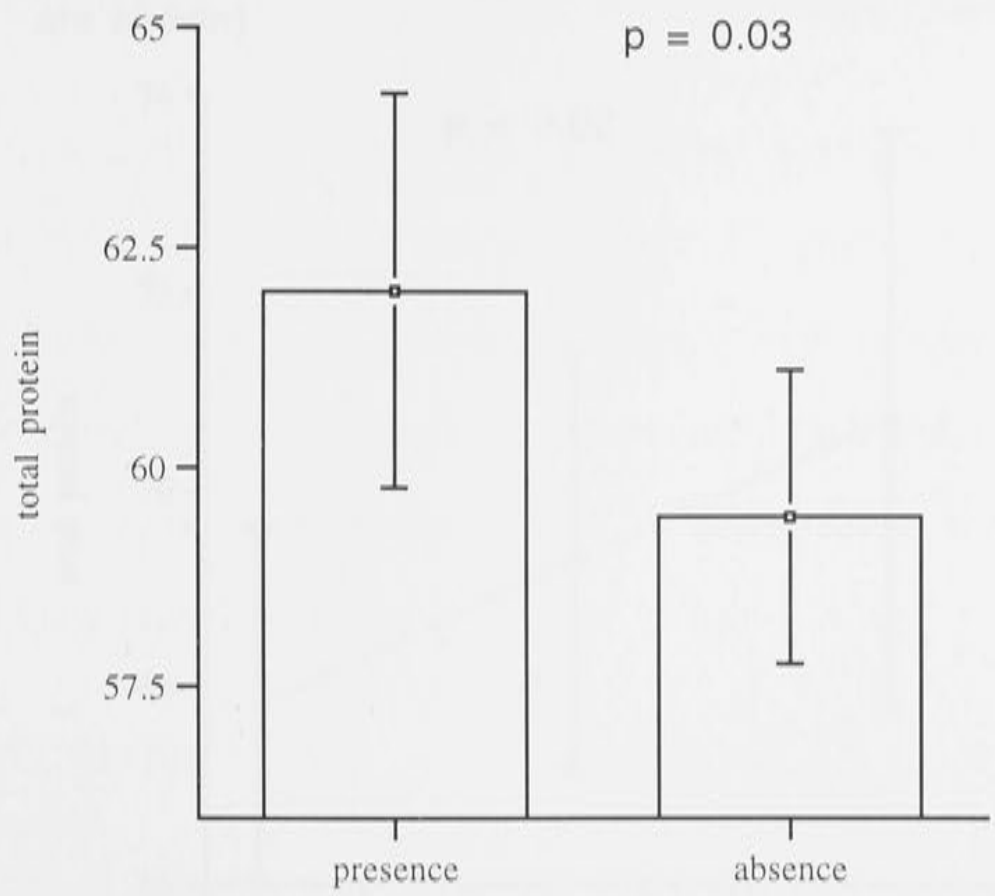


Figure 7.22 a: Graph of total serum protein levels in *T. caninus* in three States given a mean intensity of infestation with *I. trichosuri* (predicted means and approximate 95% confidence intervals are shown)

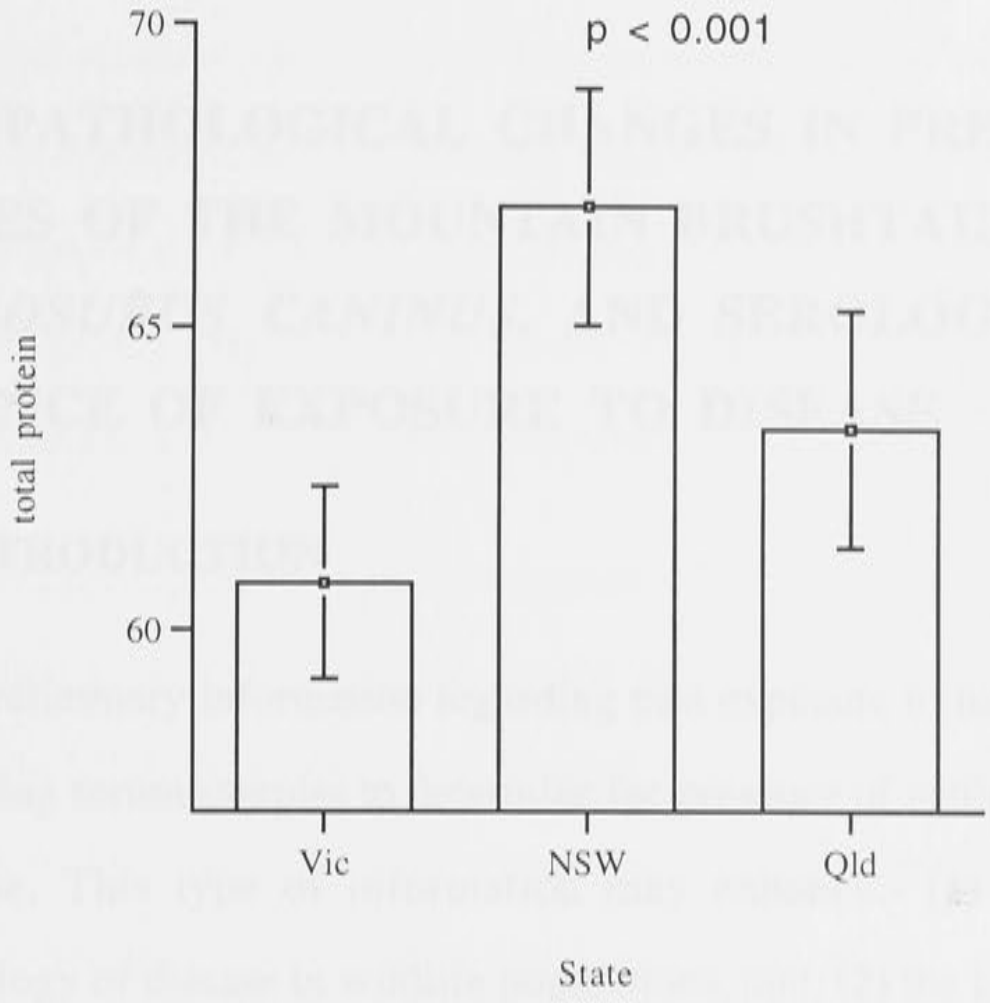
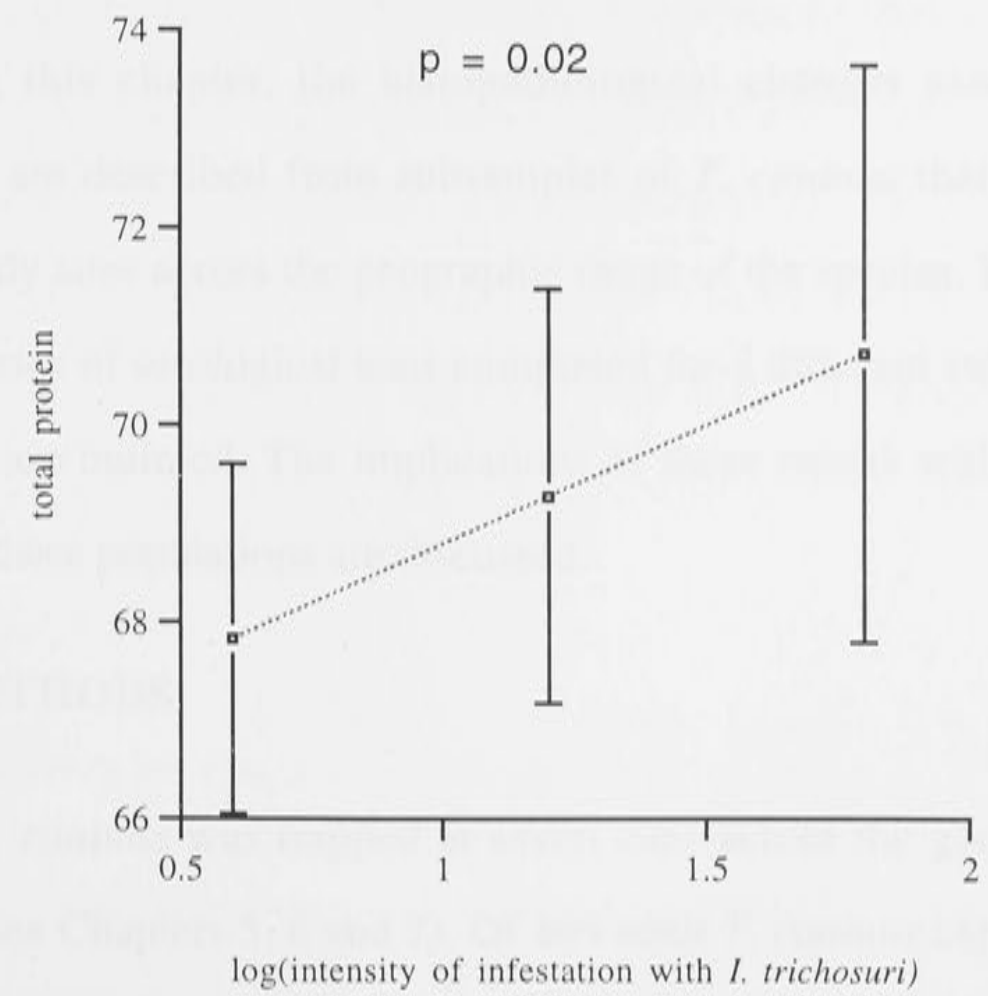


Figure 7.22 b: Graph of the relationship between total serum protein and log(intensity of infestation with *I. trichosuri)* in *T. caninus* from NSW
(predicted means and approximate 95% confidence intervals are shown)



CHAPTER 8

HISTOPATHOLOGICAL CHANGES IN PRESERVED TISSUES OF THE MOUNTAIN BRUSHTAIL POSSUM, *TRICHOSURUS CANINUS*, AND SEROLOGICAL EVIDENCE OF EXPOSURE TO DISEASE

8.1 INTRODUCTION

Preliminary information regarding past exposure to infection can be obtained by analysing serum samples to determine the presence of antibodies to specific agents of disease. This type of information may enhance:- (**1**) understanding of the epidemiology of disease in wildlife populations, and, (**2**) the identification of wildlife populations which may function as reservoirs of diseases that are transmissible to humans (Mackerras 1954; Smyth 1995). Further information on disease and the pathogenic effects of parasites on their hosts may be obtained by histopathological examination of tissue samples collected routinely during necropsy .

In this chapter, the histopathological changes associated with helminth infection are described from subsamples of *T. caninus* that were necropsied from seven study sites across the geographic range of the species. In addition, the findings from a series of serological tests completed for a different subset of *T. caninus* from each site are outlined. The implications of these results with respect to the disease status of these populations are discussed.

8.2 METHODS

T. caninus was trapped at seven sites across the geographical range of the species (see Chapters 5, 6 and 7). Of 104 adult *T. caninus* captured, 26 animals were euthanased for a detailed post mortem and parasitological examination (see Chapter

3.11, Chapter 7.3.1 and Table 7.1). Tissue samples from most organs (see Table 3.4) and any grossly visible lesions were dissected and fixed in neutral buffered 10% formalin for histopathological examination. Trimmed tissues were embedded in paraffin and sectioned at 6µm. All sections were mounted on microscope slides and stained with haematoxylin and eosin by Dorevitch Pathology of Camberwell, Victoria, prior to examination. Slides were examined under a light microscope at X40 magnification for major changes in tissue structure and architecture and obvious lesions. Unusual or abnormal areas were examined at higher magnifications.

Serum samples were collected (see Chapter 3.6) from a subset of animals from each study site for serological analyses (see Table 8.1). Samples were stored for several months at -70°C and later submitted to the Elizabeth Macarthur Agricultural Institute (EMAI), Camden, NSW, for analysis. For detection of antibodies to *Coxiella burnetii*, Coxifix® test antigen (Rhonemerieux, Lyon, France) was used in a complement fixation test. Toxolatex® (Fumouze Laboratoires, Asniéres, France) latex slides were used for detection of serum antibodies to *Toxoplasma gondii*. Testing for antibodies to *Chlamydia* spp. was completed using bovine chalmydial antigen (RBL, Armidale, Australia). All other tests were completed using protocols developed at EMAI. These tests were: a complement fixation test for *Brucella abortus*, microscopic agglutination tests (MAT) for *Leptospira interrogans* serovars *hardjo* and *pomona*, an enzyme-linked immunosorbent assay (ELISA) for the Simbu group of viruses (Akabane, Aino, Peaton, Douglas, Tinaroo, Thirmi and Facey's Paddock viruses), a gel diffusion precipitation test for adenovirus, and virus serum neutralisation titres for encephalomyocarditis virus (EMC) and Ross River Virus (RRV) (Table 8.1).

8.3 RESULTS

8.3.1 Histopathological findings

Aggregations of lymphoid cells were found adjacent to blood vessels in the lungs and livers of all *T. caninus* examined. This characteristic has been observed in

numerous other histopathological sections of lung and liver from both *T. vulpecula* and *T. caninus* without any other associated pathological changes and may be normal for *Trichosurus* spp. (D. Obendorf, pers comm.). Histopathological changes were recorded from tissues of animals infected with *Ophidascaris robertsi*, *Marsupostrongylus minesi, Sprattia venacavincola* and *Sarcocystis* sp.

Liver lesions were detected in association with third stage larvae of the nematode *O. robertsi* in the bile ducts and hepatic parenchyma of several *T. caninus* from Bulburin State Forest in central Queensland (N = 4), the Conondale Ranges in southeastern Queensland (N = 1), and at Barrington Tops in central NSW (N = 1). Hepatic cells around the periphery of these lesions showed evidence of fatty change and hydropic change. Third stage larvae of *O. robertsi* typically were surrounded by a fibrogranuloma, consisting of a mixture of eosinophils and mononuclear cells, and fibroblasts forming a loose capsule (Plates 8.1 and 8.2). Immediately adjacent to these parasites, proteinaceous and cellular debris was evident, probably due to movement and feeding activities by these nematodes. Phagocytic multinucleate giant cells also were present. These cells appeared to be in the process of engulfing and removing cellular debris. More chronic fibrogranulomatous lesions were characterised by thicker walls with evidence of reactive histiocytosis (macrophage proliferation). Depleted lymphoid follicles were present in the mesenteric lymph nodes of affected animals. This may have been due to chronic production of immunoglobulins in response to the presence of the *O. robertsi* larvae in the liver (D. Obendorf, pers comm.).

The lungs of almost all necropsied *T. caninus* showed evidence of histopathological changes. Foamy macrophages with mononuclear cell infiltrates were present in the alveoli of most animals, both in the presence and absence of *M. minesi,* as determined from both necropsy and histological examination of lung tissue. In lung sections from some animals, numerous examples of developmental stages of eggs, larvae and adult worms of *M. minesi* were present (Plate 8.3). This parasite is oviparous (Spratt 1979) (Plate 8.4), with embryonation of eggs occurring in the lung

tissue. Eggs were present at various stages of embryonation and some larvae were evident coiled within their vitelline membranes (Plate 8.5). A transverse section of one adult *M. minesi* clearly showed that the gut contained red blood cells, implying that these parasites are haematophagous. Histopathological changes associated with the presence of *M. minesi* appeared to be related to the intensity of infection. At higher intensities of infection, *M. minesi* appeared to cause an interstitial pneumonia at the alveolar level, characterised by eosinophil and mononuclear cell infiltrations. Considerable atelectasis of lung tissue was evident, with areas of emphysema ringing areas damaged by parasites (Plate 8.4). There was no evidence of bronchopneumonia.

In one *T. caninus* from Cambarville in central Victoria, a transverse section of a female lungworm containing numerous larvae was found in an area of relatively normal lung (Plate 8.6). This was the first record of a metastrongyloid nematode from *T. caninus* at this site. Viviparity (giving birth to free-larvae) in this nematode species, indicated that it was not *M. minesi*, which is oviparous (Spratt 1979).

Multiple small pale foci were visible grossly throughout the lung parenchyma of three *T. caninus* from Cambarville in central Victoria. These foci were characterised histologically by infiltrations of eosinophils and some macrophages, into the airways to form eosinophilic granulomas. Perivascular cuffing of eosinophils around venules was also present (Plates 8.7 and 8.8). These lesions were typical of those evident in cases of a hypersensitivity or allergic pneumonia. Within some of these granulomas, a small amount of unidentifiable degenerate material was present (Plate 8.9), which was probably a type of foreign body. In more chronic advanced stages of this granulomatous lesion, eosinophils were replaced by mononuclear cells. In addition, fibrosis of the pulmonary parenchyma was organised and extensive, with only limited functional respiratory tissue remaining (Plate 8.9). Advanced lesions represented an end-stage pneumonitis.

A single microfilaria was detected in a glomerulus within the kidney of one *T. caninus* from Barrington Tops in central NSW (Plate 8.10). Numerous microfilariae

of the filarioid nematode *Sprattia venacavincola* were detected and identified (see Spratt and Varughese 1975) from the peripheral blood of this animal using the blood concentration technique described in Chapter 3.9). Therefore it was considered likely that the microfilaria found in this histological section was from *S. venacavincola.* Clusters of red blood cells were associated with the presence of this microfilaria. Microarchitectural changes were also evident, including dilation of the capillary bed in the glomerulus. This was probably associated with circulatory alteration due to the presence of the microfilaria.

A cystic structure was detected in the muscularis mucosa of the tongue of one *T. caninus* from Bulburin State Forest in central Queensland. This structure contained small zoites with fimbriae evident around the cyst wall, and was probably a thick-walled sarcocyst associated with infection with an unidentified species of the protozoan parasite *Sarcocystis* (Plates 8.11 and 8.12) (Munday *et al.* 1978). There was no cellular reaction to the presence of this sarcocyst.

No histopathological changes were detected in association with the presence of *Paraustrostrongylus trichosuri, Parastrongyloides trichosuri* or *Adelonema trichosuri.*

8.3.2 Serological analyses

The results of the serological tests completed for 40 *T. caninus* from the seven study sites are given in Table 8.1. For all *T. caninus* tested, insignificant antibody titres were recorded to *B. abortus*, *Chlamydia* spp., *L. hardjo* and *L. pomona*. Values for significant titres for these tests vary between laboratories. Titres are generally interpreted in association with clinical signs of disease and may differ between species. A positive titre for EMC was recorded from one *T. caninus* from the Conondale Ranges in southeastern Queensland. Positive titres for RRV were detected in *T. caninus* from Bulburin State Forest (N=1) and the Conondale Ranges (N=3) in Queensland, Byrangery Reserve (N=2) in northeastern NSW and Bellbird (N=2) in eastern Victoria. Negative titres for RRV were recorded for *T. caninus* from the other

study sites. Tests for Simbu Virus and Adenovirus antibodies were negative. Titres for *T. gondii* were low. CFT titres for Q fever are difficult to interpret (Worswick and Marmion 1992), but titres of eight or more probably indicate past exposure to the disease agent, *C. burnetii.*

8.4 DISCUSSION

8.4.1 Histopathology

The presence of larvae of the ascaridoid nematode *O. robertsi* was associated with significant pathological changes in the liver parenchyma. Presidente *et al.* (1982) described the histopathological changes associated with the presence and migration of these larvae, including focal eosinophilic cholangiohepatitis and dilatation of the affected bile ducts. Interestingly, in this study of *T. caninus* from seven study sites, those animals with high intensities of infection with *O. robertsi*, did not exhibit serum elevation of liver-associated metabolites and enzymes (ALT, GGT, ALP and bilirubin). Bilirubin and ALT may be elevated when the liver parenchyma is damaged, and elevation of ALP is generally associated with pathology or obstruction of the bile ducts (Bush 1991; Duncan *et al.* 1994). The lack of change in these enzymes and metabolites in *T. caninus* infected with *O. robertsi* may be due to:- **(1)** chronic infection with third stage larvae of *O. robertsi*, in which the parasite becomes encysted within a fibrous capsule, causing little ongoing damage to hepatic or bile duct cells; and **(2)** insensitivity of serum enzyme tests to low grade or localised tissue damage.

Parasitic pneumonia was detected in association with the presence of *M. minesi* in the lungs of several *T. caninus*. Similar lesions have been described from swamp wallabies, *Wallabia bicolor* infected with *M. dorrigoensis* and *M. wallabiae* (Beveridge *et al.* 1985). In other hosts, *Marsupostrongylus* spp. occur in the alveoli and bronchioles, causing an interstitial pneumonia (Beveridge 1978). In a koala, *Phascolarctos cinereus*, infected with a *Marsupostrongylus* sp., degenerating larvae and remnants of nematode cuticle were found in the alveoli, with infiltration of

eosinophils, neutrophils and some giant cells (McColl and Spratt 1982). Severe infections with *Marsupostrongylus* spp. may cause impaired respiratory function, eg. in *W. bicolor*, from the Bonang area of Victoria (Presidente, in Beveridge 1978). However, in this study of *T. caninus*, *M. minesi* did not appear to be present at sufficiently high intensities to clinically affect host respiration.

End-stage pneumonitis with chronic active fibrosis and inflammatory cell infiltrates was described from the lungs of two *T. caninus* at Cambarville in central Victoria. This massive tissue reaction appeared to be due to the presence of an unidentified foreign body, which was in the advanced stages of degradation. In less chronic lesions, the cellular response to this foreign body was dominated by eosinophils, which often are associated with parasitic invasion or allergic responses (Rothwell and Dineen 1972). It is possible that lungworms from another host species were attempting to establish in the lungs of *T. caninus*. In host reactions to nematode invasions of the lungs, the cuticle is usually the last anatomical structure of the parasite to be destroyed by the host immune response and is a good indication of a parasitic infection (Spratt, pers. comm; see McColl and Spratt 1982). However, no degenerative material representative of nematode cuticle was detected in these sections.

A thick-walled sarcocyst was detected in the tongue of one *T. caninus* in this study. *Sarcocystis* spp. are protozoan parasites that are sought rarely and thus detected only occasionally in skeletal muscle tissues of marsupials (Munday *et al.* 1978). In *Trichosurus* spp., sarcocysts are typically thin-walled and contain small zoites (Munday *et al.* 1978); this is the first record of a thick-walled form from *T. caninus*.

Histopathological examination of tissue sections in this study indicated that *O. robertsi* and *M. minesi* caused damage to tissues of *T. caninus*. No histopathological changes were recorded in association with the presence of other helminth parasites from the array of animals necropsied across the seven study sites. *Paraustrostrongylus trichosuri* is usually found tightly coiled around the base of villi in the proximal small intestine (Mawson 1973; Smales and Mawson 1978; Presidente 1984). Presidente *et*

al. (1982) reported the presence of swollen enterocytes and mild mononuclear cell infiltration where the paired lateral extensions of the body of this parasite contacted the villi. In this thesis, samples of small intestine were collected from most *T. caninus* (N=15) that were infected with *Paraustrostrongylus trichosuri* and/or *Parastrongyloides trichosuri*. These tissue samples were collected after the removal of parasites and before scraping of the mucosa, however, no pathological changes were evident. There were no pathological changes in sections of caecum in association with the presence of the oxyurid nematode *A. trichosuri*. This parasite occurs in the caecal contents and appears to cause little damage to host tissues.

There was no histopathological evidence of other disease processes in the sections examined from *T. caninus* in this study. Culture of tissues or body fluids may be used to test for specific disease agents. However, no culturing was done in this study.

8.4.2 Serology

8.4.2.1 Leptospirosis

Insignificant titres to *L. interrogans* serovars *hardjo* and *pomona* were recorded for all *T. caninus* that were tested in this study. Antibodies to the *L. interrogans* serovars *balcanica* and *hardjo* cross react in agglutination tests (Durfee and Presidente 1979a, 1979b). In those *T. vulpecula* with *L. hardjo* antibodies, the serovar *L. balcanica* has been routinely isolated (Durfee and Presidente 1979a, 1979b). Hence, microscopic agglutination tests for *L. hardjo* commonly have been used to detect antibodies to *L. balcanica* in *T. vulpecula* (Durfee and Presidente 1979a, 1979b, 1979c). The absence of titres against *L. hardjo* in *T. caninus* is surprising, given that infection of *T. vulpecula* with *L. balcanica* is considered endemic in Victoria (Durfee and Presidente 1977, 1979a), NSW (Milner *et al.* 1981) and New Zealand (Hathaway *et al.* 1978).

T. caninus spends a considerable amount of time foraging on the ground (Chapter 2.6; Seebeck *et al.* 1984), hence contact with *L. balcanica* would be likely if it were present in the local environment. It is possible that *T. caninus* may become infected with *L. balcanica* in regions where the species occurs in sympatry with *T. vulpecula*. However, a previous survey of *T. caninus* at Clouds Creek, also failed to detect antibodies to *L. hardjo* from 55 animals, some of which occurred in sympatry with *T. vulpecula* (Durfee and Presidente 1979a). These authors reported lesions of focal interstitial nephritis in the kidneys of three *T. caninus*, which were consistent with *L. balcanica* infection, suggesting that *T. caninus* may contract this disease. In addition, three *T. vulpecula* collected from the same region (Clouds Creek) had antibodies to *L. hardjo* (Durfee and Presidente 1979a). No lesions suggestive of nephritis were found in any of the sections from *T. caninus* that were examined for this thesis.

8.4.2.2 Ross River Virus (RRV)

In this study, positive titres to RRV were recorded in *T. caninus* from several study sites; eastern Victoria (Bellbird), northeastern NSW (Byrangery Reserve), and southeastern and central Queensland (Conondale Ranges and Bulburin State Forest). These titres were significant and indicate widespread exposure to this virus. This provides evidence that *T. caninus* may function as a reservoir for infection and amplification of this disease.

Ross River virus (RRV) is an arbovirus that is transmitted by mosquitoes. It was originally isolated at Townsville and occurs in the northern parts of Northern Territory, and the Kimberley region of Western Australia as well as southeastern W.A. In humans, it causes epidemic polyarthritis (Fraser *et al.* 1992). Native and domestic mammals are the vertebrate hosts for this virus (Vale *et al.* 1991), and positive results for antibodies to RRV have been recorded from *T. vulpecula* in NSW, Tasmania (Gard *et al.* 1973; Presidente 1984) and Kangaroo Island, South Australia (O'Callaghan and Moore 1986).

8.4.2.3 Encephalomyocarditis virus (EMC)

EMC viruses are ubiquitous in the environment and have been isolated from a variety of mammals, birds and insects (Murnane 1982). This disease has zoonotic significance, and rodents are probably the major reservoir of infection (Murnane 1982). The only positive titre to this virus was detected in a *T. caninus* in the Conondale Ranges in southeastern Queensland. Notably, significant problems were encountered with trapping *T. caninus* at this study site due to interference with traps by bush rats, *Rattus fuscipes*. This problem was not experienced at the other study sites. It is possible that this native rodent may become infected with EMC virus, and at the high densities of *R. fuscipes* recorded at this study site, *T. caninus*, which also feeds on the ground (Seebeck *et al.* 1984; Chapter 2.4) may have been exposed to this virus.

8.4.2.4 Q fever

Q fever is a zoonotic disease that occurs throughout Australia, causing fever and pneumonia in infected humans (Roberts 1992). In Australia, cattle are the main domestic host for the causative agent, *C. burnetii,* and northern brown bandicoots, *Isoodon macrourus,* are the main wildlife reservoir (Smith and Derrick 1940). However, antibodies have been found, or infection has been established experimentally, in a broad range of native animals (Stevenson and Hughes 1988; Williams and Sanchez 1994), including cats and sheep. The tick *Haemaphysalis humerosa*, is the main vector for *C. burnetii* (Smith and Derrick 1940), although *Ixodes holocyclus* also has been identified as a possible vector (Derrick 1944). There are no records of ticks of the genus *Haemaphysalis* from *T. caninus.* However, in this study, *I. holocyclus* was recorded from *T. caninus* at all study sites except Cambarville in central Victoria (see Chapter 7). Therefore, if *I. holocyclus* is a vector for *C. burnetii, T. caninus* may be exposed to C. *burnetii* through infestation with this tick species. In addition, *C. burnetii* is highly infectious and may be transmitted percutaneously to uninfected animals by contact with tick faeces alone (Smith 1940).

Interpretation of Q fever CFT titres may vary between laboratories. Opinions vary as to what constitutes a significant titre (Worswick and Marmion 1992). Low titres for Q fever (<8, 8 and 16) were recorded from *T. caninus* in this study (see Table 8.1). Titres of eight and 16 may indicate past exposure to *C. burnetii,* however, titres <8 are difficult to interpret and are probably negative results.

8.4.2.5 Toxoplasmosis

T. gondii is a protozoan parasite which may cause disease in a wide range of domestic and native animals, particularly under circumstances of stress or overcrowding (Stevenson and Hughes 1988). The definitive host of this parasite is the domestic and feral cat, *Felis catus*, and infection of other animals, including humans, usually occurs by ingestion of cysts that are shed in the faeces of infected cats (Stevenson and Hughes 1988). In this study of *T. caninus*, antibody titres for toxoplasmosis were low (see Table 8.1). Interpretation of these results is difficult as low titres may indicate false or weak positive reactions, or past exposure to disease with declining antibody titres. Feral cats are present throughout the distribution of *T. caninus*, and it is possible that *T. caninus* may come into contact with cat faeces. However, the results of this study indicate that it is unlikely that any of the animals tested were recently exposed to *T. gondii.*

8.4.3 Limitations of serological tests to detect antibody titres

Interpretation of results of serological tests may at times be equivocal and difficult to interpret. A positive result may indicate:- (**1**) a present and current infection, (**2**) a previous infection to which the host is now immune, (**3**) a cross-reaction with shared antibody from another infection, or, (**4**) presence of antibody transferred from mother to young. Alternatively, a negative result to a serological test may indicate:- (**1**) the individual is not, and never was, infected, (**2**) recent infection to which antibody has not yet developed, (**3**) the host has been previously infected, but immunity was of short term duration and antibody is no longer present, or, (**4**) the

host has been infected, but could not produce antibody. Therefore, to accurately determine the disease status of animals, longitudinal studies are required, involving repeat sampling over time of specific individuals within the same populations (Vale *et al.* 1991). Data derived from long term serological studies of exposure to disease, coupled with investigations of animal survival and reproduction, may provide information of the effect of disease on host abundance and population dynamics (Anderson 1996). Despite this, initial screening tests, such as those completed in this study, provide useful information on the disease agents to which a population of animals may have been exposed and identify suitable populations for future or ongoing studies.

8.5 CONCLUSIONS

Histopathological changes were detected in tissue sections from *T. caninus* infected with *O. robertsi* and *M. minesi*. Unidentified foreign bodies in the lungs of two animals from the Cambarville population were associated with fibrogranuloma formation and major changes in tissue architecture, including massive loss of functional respiratory tissue. Further study is required to identify the causative agent, which may be a parasite, and to document its effects on this host. An examination of the effects of parasites on their hosts at the population level, rather than the individual level, is required to further investigate the role of parasites in host population dynamics and regulation (Grenfell and Gulland 1996) (see Chapters 9 and 10).

Serological tests completed for *T. caninus* from several study sites, indicated that exposure to various disease agents was low. Negative titres were recorded for many of the tests that were completed. The finding of positive titres to RRV in animals from several populations indicates that *T. caninus* may be a wildlife reservoir for this virus. There was no evidence of clinical signs associated with any of the disease agents for which animals tested positive.

Table 8.1: Serological test results for *T. caninus* from seven study sites across the geographic range of the species

Animal	*Brucella abortus* Complement fixation test titre	*Chlamydia* Complement fixation test titre	*Leptospira hardjo* Microscopic agglutination test titre	*Leptospira pomona* Microscopic agglutination test titre	Q fever Complement fixation test titre	*Toxoplasma* Latex agglutination titre	Simbu Enzyme-linked immunosorbance assay	Adenovirus Gel diffusion precipitation test grade	Encephalomyocarditis Virus Virus neutralisation test titre-serum at 1:10	Ros River Virus Virus neutralisation test titre-serum at 1:10
Cambarville (central Victoria)										
C3	<4	<8	<25	<25	8	<4	NEG	NEG	NEG	NEG
C10	<4	<8	<25	<25	<8	<4	NEG	NEG	NEG	NEG
C15	<4	<8	<25	<25	<8	<4	NEG	NEG	NEG	NEG
C28	AC	<8	<25	<25	8	<4	NEG	NEG	NEG	NEG
C32	AC	<8	<25	<25	8	<4	NEG	NEG	NEG	NEG
C36	<4	<8	<25	<25	<8	<4	NEG	NEG	NEG	NEG
Bellbird (eastern Victoria)										
BB4	AC	<8	<25	<25	<8	<4	NEG	NEG	NEG	NEG
BB25	<4	<8	<25	<25	<8	<4	NEG	NEG	NEG	16
BB40	<4	<8	<25	<25	<8	<4	NEG	NEG	NEG	NEG
BB44	AC	<8	<25	<25	<8	<4	NEG	NEG	IS	NEG
BB45	AC	<8	<25	<25	<8	<4	NEG	NEG	NEG	256
Barrington Tops (central NSW)										
BTP7	<4	<8	<25	<25	<8	4	NEG	NEG	NEG	NEG
BTP10	<4	<8	<25	<25	IS	<4	NEG	NEG	NEG	NEG
BTP12	<4	<8	<25	<25	<8	<4	NEG	NEG	NEG	NEG
BTP14	AC	<8	<25	<25	16	<4	NEG	NEG	NEG	NEG
BTP15	<4	<8	<25	<25	<8	4	NEG	NEG	NEG	NEG
BTP19	AC	<8	<25	<25	8	<4	NEG	NEG	NEG	NEG
BTP21	AC	<8	<25	<25	8	<4	NEG	NEG	NEG	NEG
Whian Whian State Forest (northeastern NSW)										
WW1	<4	<8	<25	<25	IS	IS	NEG	NEG	IS	NEG
WW4	AC	<8	<25	<25	IS	IS	NEG	NEG	IS	NEG
WW6	AC	<8	<25	<25	IS	IS	NEG	NEG	NEG	NEG

Table 8.1: continued

Animal	*Brucella abortus* CFT titre	*Chlamydia* CFT titre	*Leptospira hardjo* MAT titre	*Leptospira pomona* MAT titre	Q fever CFT titre	*Toxoplasma* Latex agglutination titre	Simbu ELISA	Adenovirus GDPT grade	EMC VNT titre-serum at 1:10	RRV VNT titre-serum at 1:10
Byrangery Reserve (northeastern NSW)										
BR1	AC	<8	<25	<25	8	<4	NEG	NEG	NEG	NEG
BR2	AC	<8	<25	<25	8	<4	NEG	NEG	NEG	128
BR3	AC	<8	<25	<25	8	4	NEG	NEG	NEG	512
BR5	AC	<8	<25	<25	16	4	NEG	NEG	NEG	NEG
Conondale Ranges (southeastern NSW)										
CD3	AC	<8	<25	<25	<8	<4	NEG	NEG	NEG	256
CD4	<4	<8	<25	<25	<8	4	NEG	NEG	NEG	>512
CD6	AC	<8	<25	<25	<8	4	NEG	NEG	NEG	NEG
CD8	AC	<8	<25	<25	<8	4	NEG	NEG	IS	positive
CD9	<4	<8	<25	<25	IS	IS	NEG	NEG	NEG	NEG
CD10	<4	<8	<25	<25	<8	<4	NEG	NEG	positive 10	NEG
CD11	<4	<8	<25	<25	IS	IS	NEG	NEG	NEG	NEG
Bulburin State Forest (central Queensland)										
BSF5	<4	<8	<25	<25	<8	<4	NEG	NEG	NEG	NEG
BSF6	AC	<8	<25	<25	8	<4	NEG	NEG	NEG	NEG
BSF7	AC	<8	<25	<25	<8	<4	NEG	NEG	IS	NEG
BSF9	AC	<8	<25	<25	<8	4	NEG	NEG	NEG	512
BSF11	AC	<8	<25	<25	IS	IS	NEG	NEG	NEG	NEG
BSF12	AC	<8	<25	<25	<8	4	NEG	NEG	NEG	NEG
BSF13	AC	<8	<25	<25	IS	IS	NEG	NEG	NEG	NEG

AC = anticomplementary
NEG = negative
IS = insufficient serum for test

Plate 8.1: Transverse section of a third stage larva of the ascaridoid nematode *Ophidascaris robertsi* in the liver of *Trichosurus caninus* from Barrington Tops (central NSW) showing proteinaceous and cellular debris in the immediate vicinity of the parasite, and the formation of fibrous tissue walling off the parasite from the surrounding hepatic parenchyma. H & E. (X160 magnification).

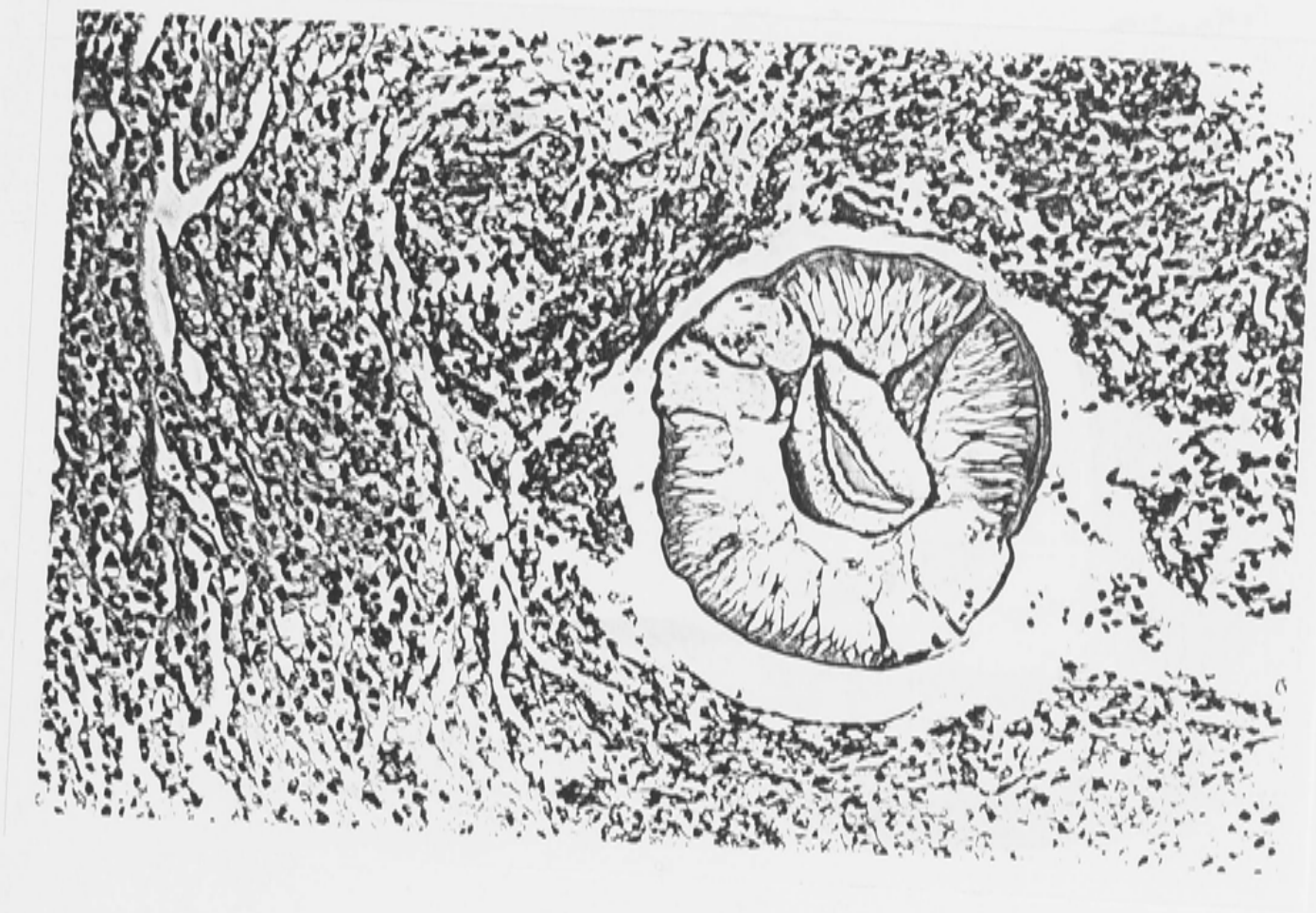


Plate 8.2: Transverse section of a third stage larva of the ascaridoid nematode *Ophidascaris robertsi* in the liver of *Trichosurus caninus* from Barrington Tops (central NSW) showing the fibrous capsule surrounding the parasite, with infiltration of mononuclear cells. H & E. (X400 magnification).

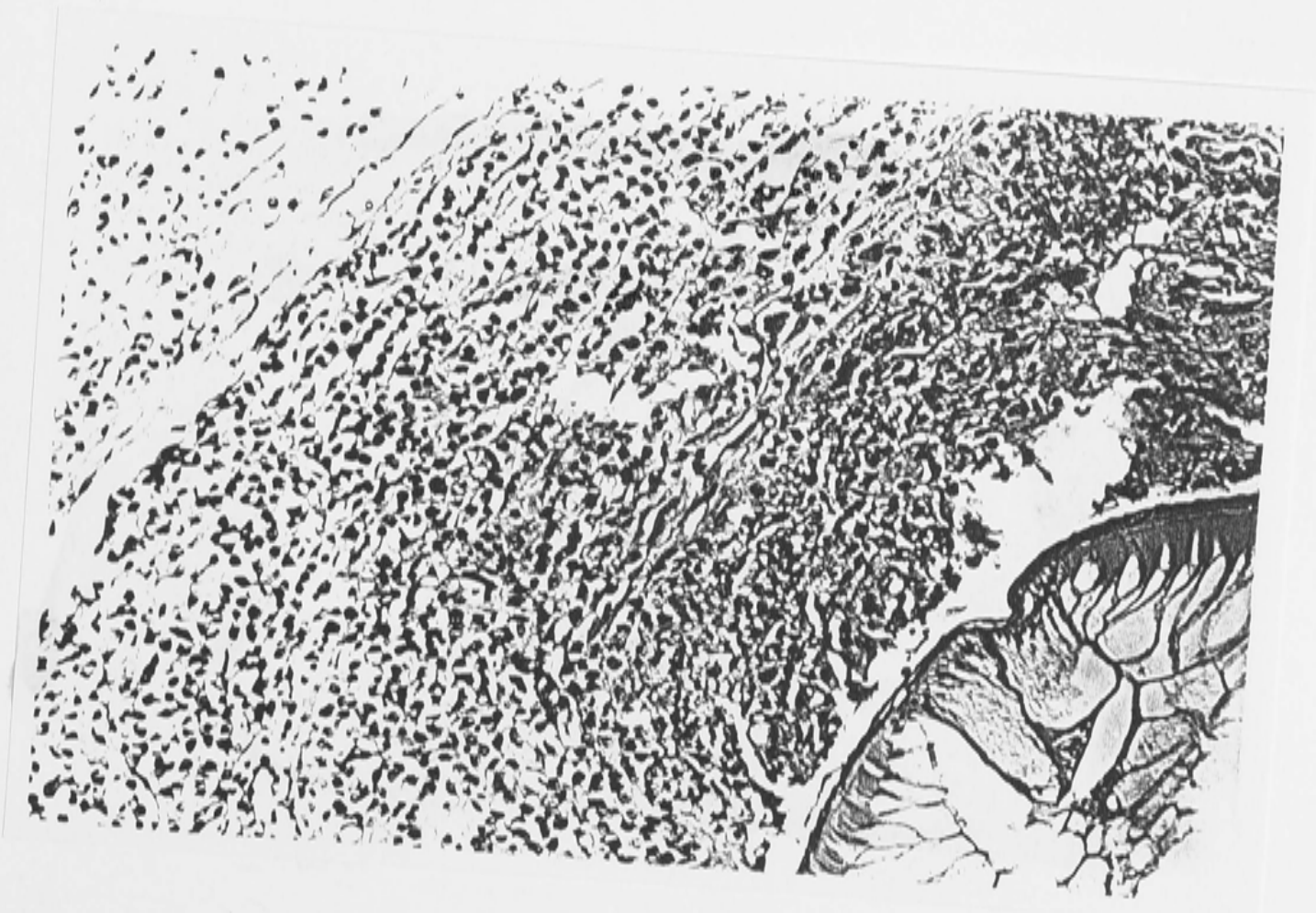


Plate 8.3: Longitudinal and transverse sections of *Marsupostrongylus minesi* in a section of lung tissue from *Trichosurus caninus* from Barrington Tops (central NSW), showing eggs at various stages of embryonation, and showing eggs, surrounded by voluminous vitelline membrane, commencing embryonation. H & E. (X400 magnification)

Plate 8.4: Transverse section of the metastrongyloid nematode *Marsupostrongylus minesi* in a section of lung tissue from *Trichosurus caninus* from Barrington Tops (central NSW), showing unembryonated eggs *in utero*. Note that there is some thickening of alveolar septae, with infiltration of mononuclear cells, and pathological emphysema adjacent to the parasite. H & E. (X400 magnification).

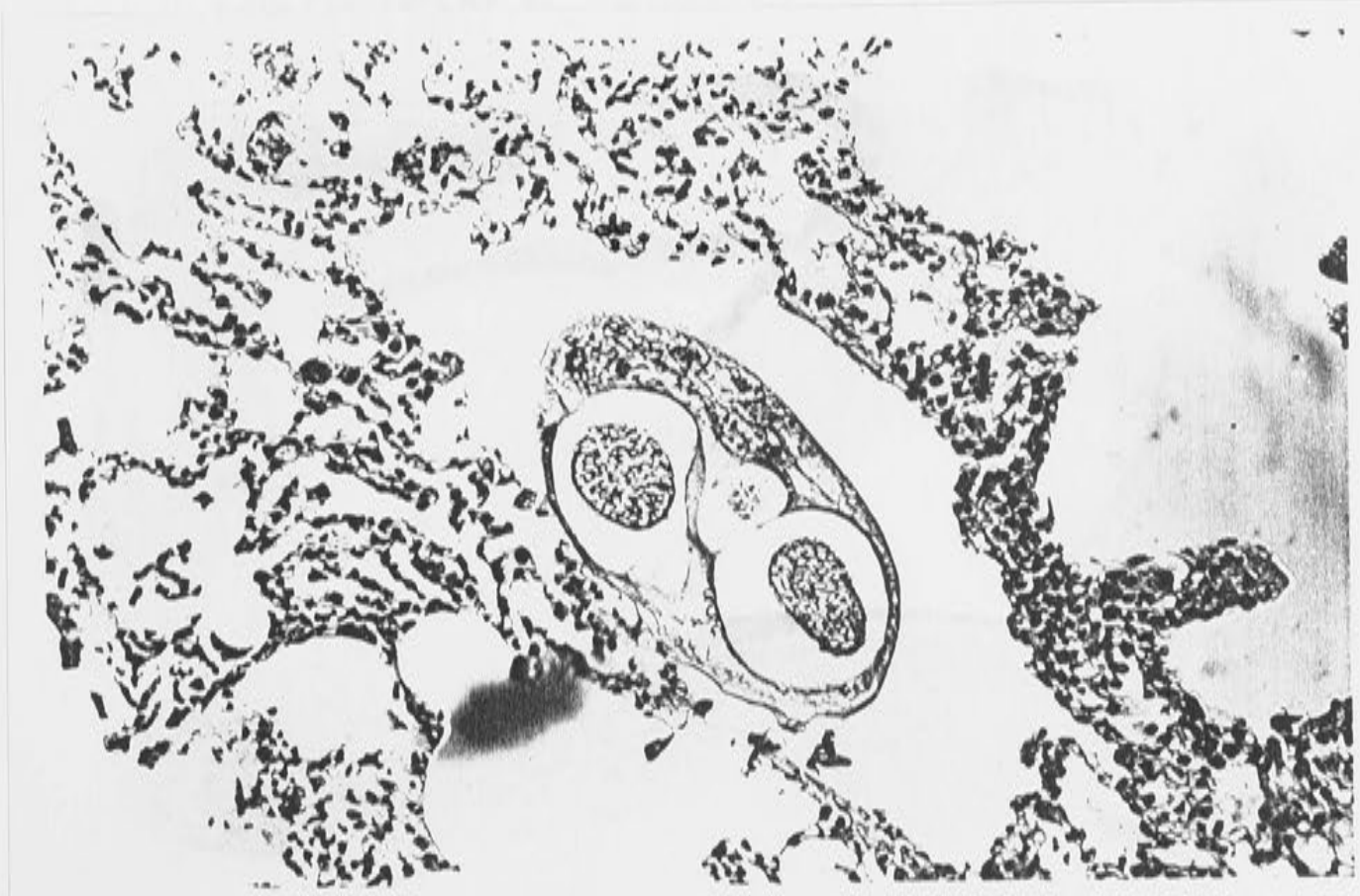


Plate 8.5: Section of lung from *Trichosurus caninus* from Barrington Tops (central NSW) showing eggs of *Marsupostrongylus minesi* which have embryonated to fully-formed larvae coiled within their vitelline membranes. H & E. (X160 magnification).

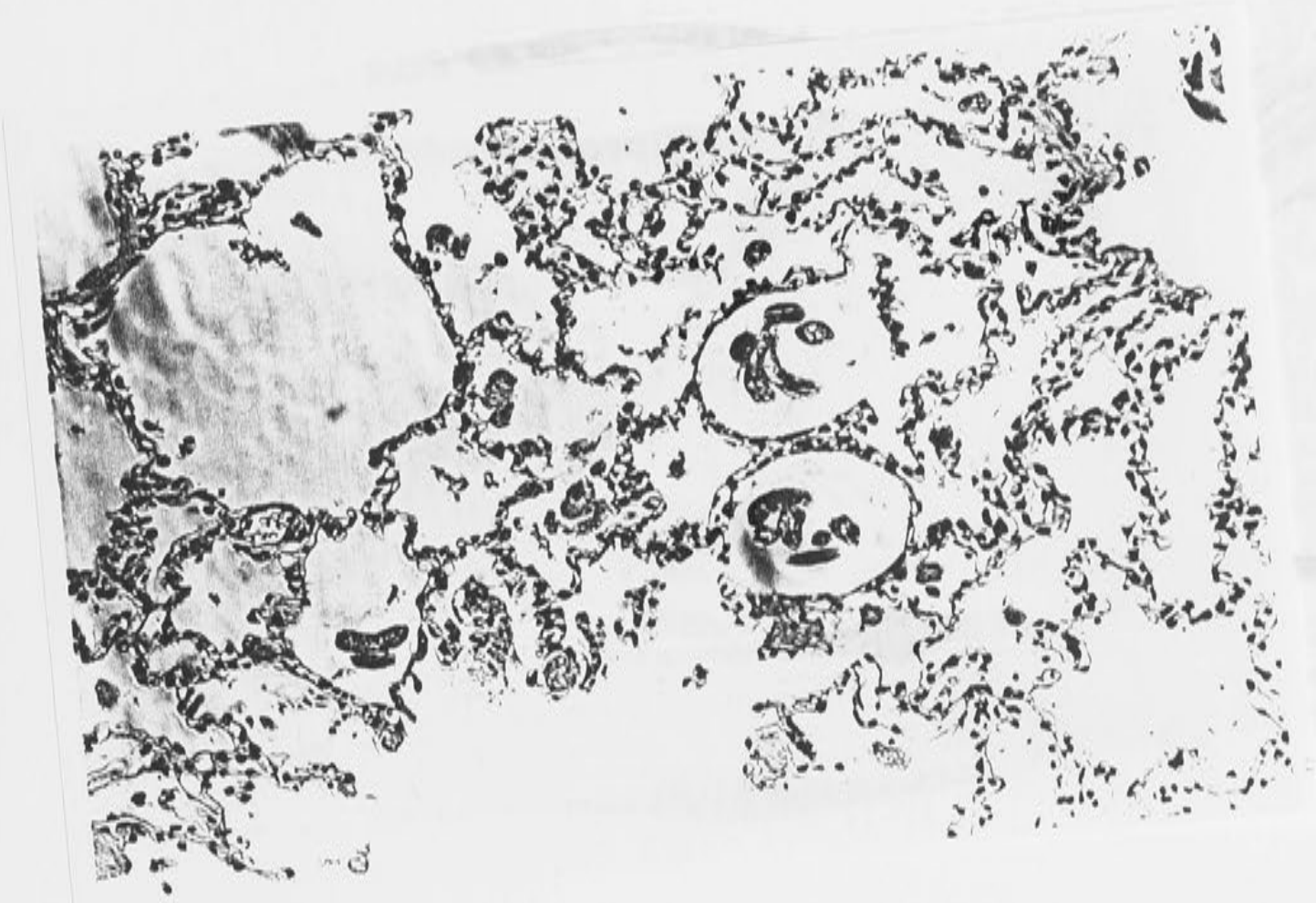


Plate 8.6: Transverse section of an unidentified viviparous female metastrongyloid nematode in a section of lung from *Trichosurus caninus* from Cambarville (Victoria) showing larvae *in utero*. H & E. (X160 magnification).

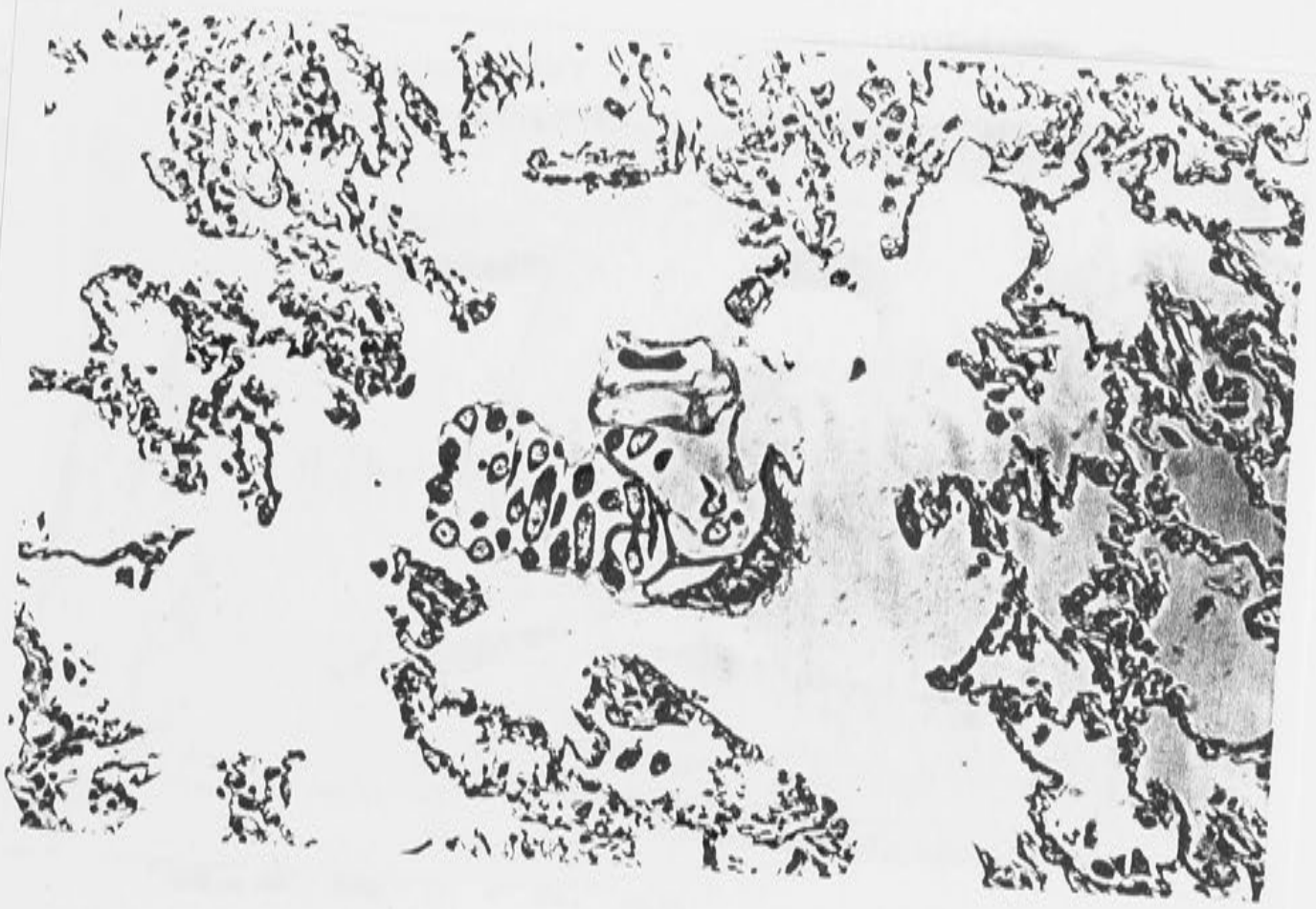


Plate 8.7: Section of lung from *Trichosurus caninus* from Cambarville (Victoria) showing perivascular cuffing of eosinophils around a venule. Note infiltration of mononuclear cells and early fibrosis in the pulmonary parenchyma. H & E. (X160 magnification).

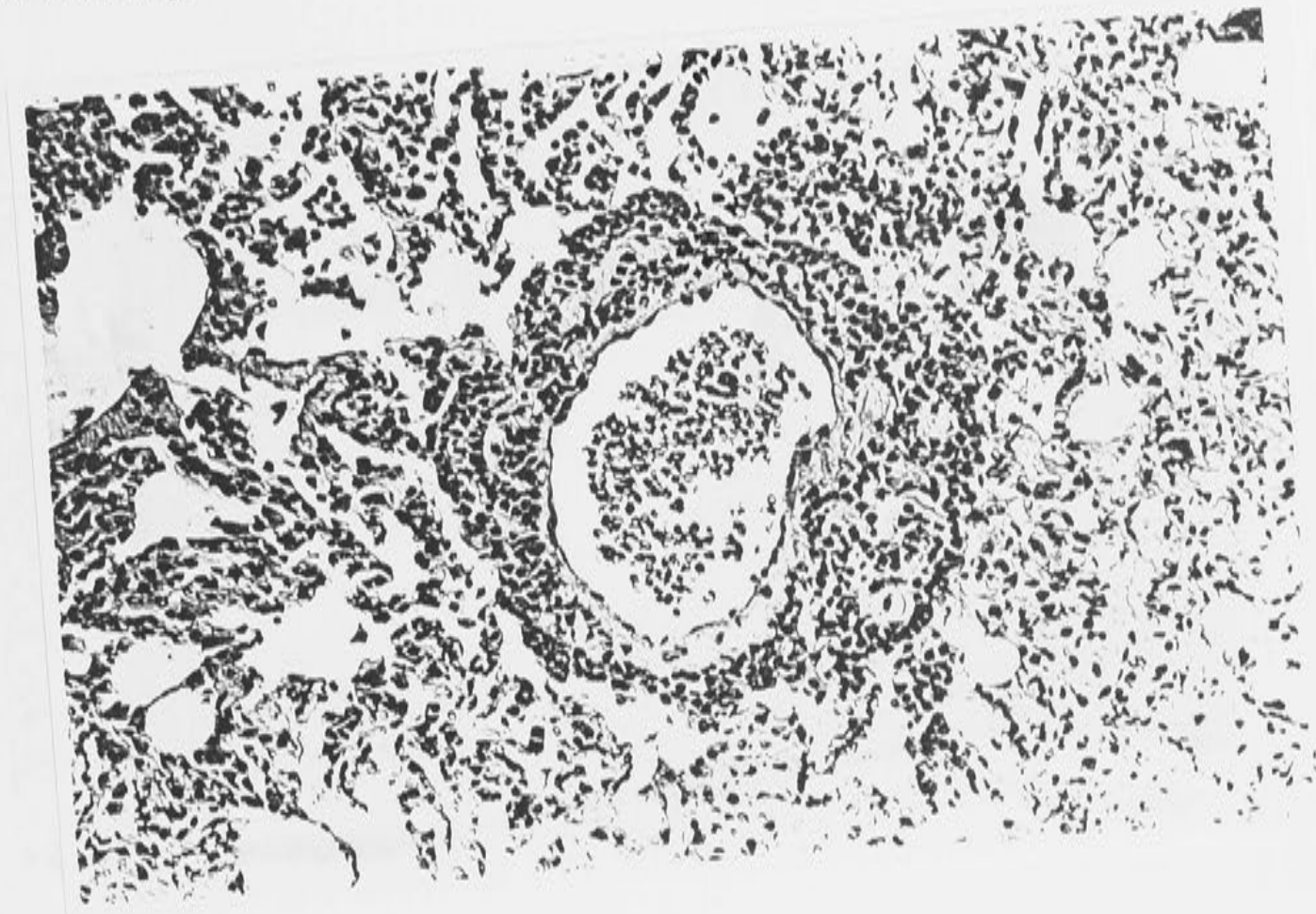


Plate 8.8: Section of lung from *Trichosurus caninus* from Cambarville (Victoria) showing perivascular cuffing of eosinophils around a venule. H & E. (X400 magnification).

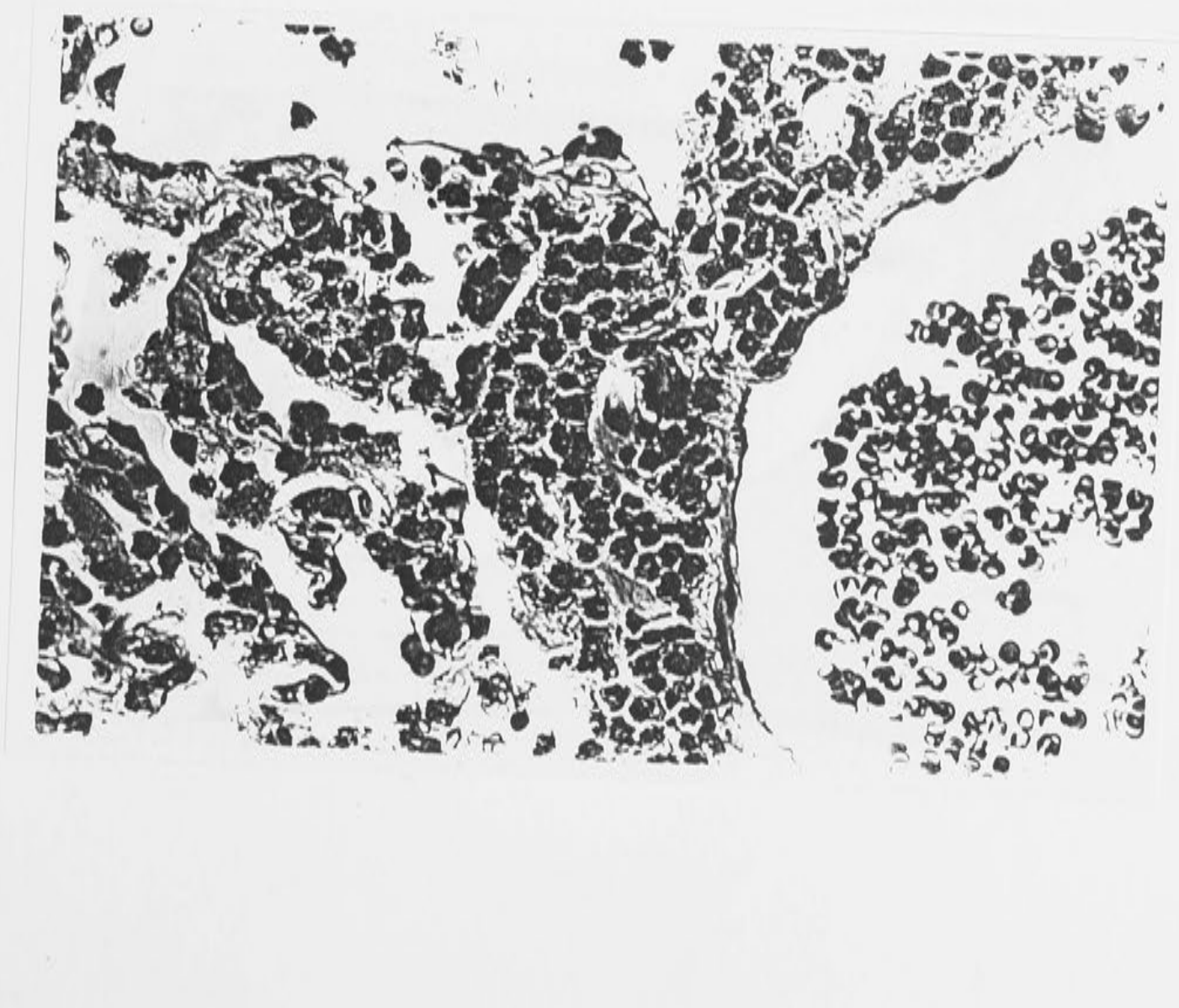
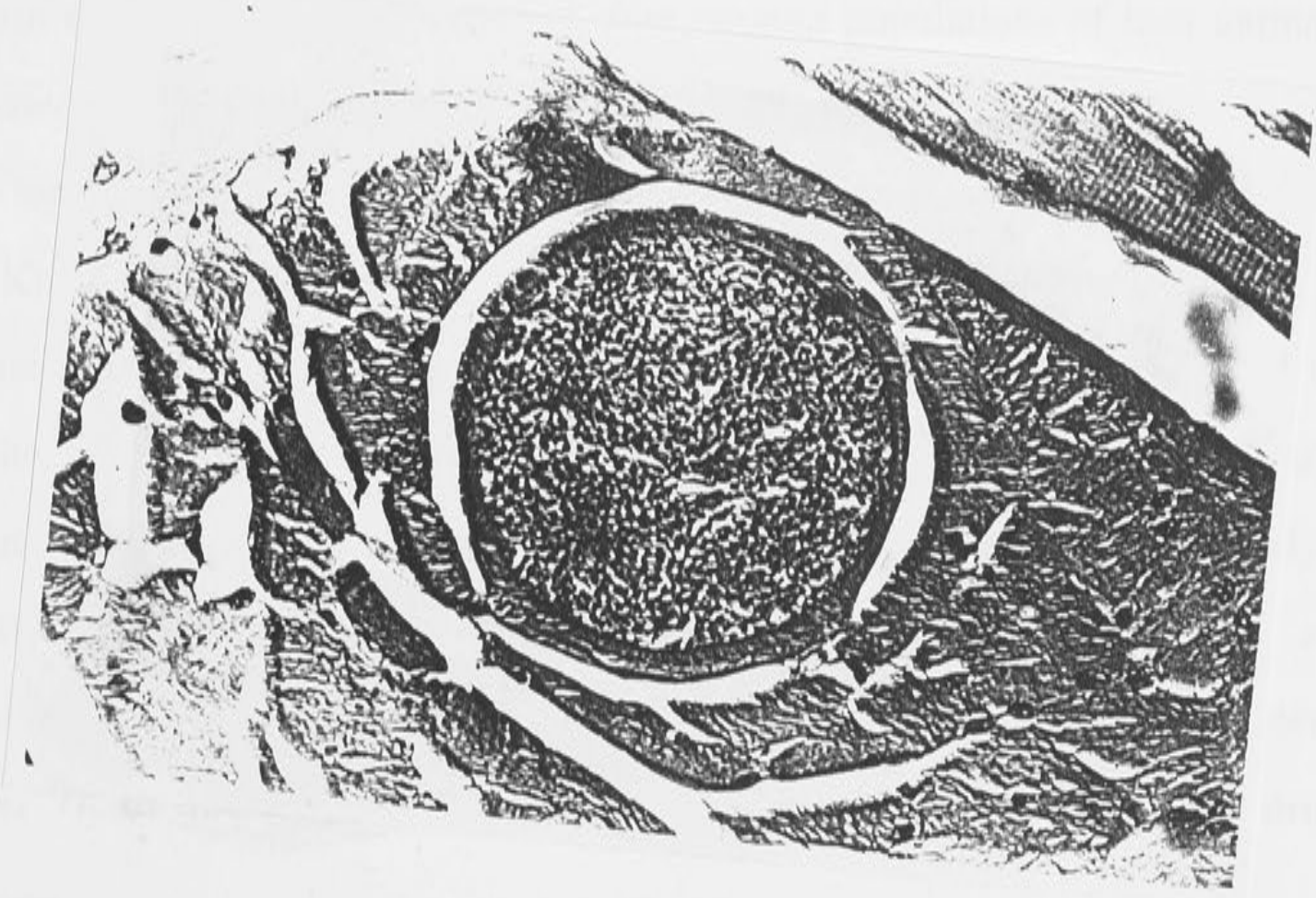


Plate 8.9: Section of lung from *Trichosurus caninus* from Cambarville (Victoria) with end-stage pneumonitis, showing massive fibrosis and loss of pulmonary parenchyma. Some unidentifiable degenerating material is visible. H & E. (X160 magnification).

Plate 8.10: Section of a kidney from *Trichosurus caninus* from Barrington Tops (central NSW), showing a longitudinal section of a microfilaria of *Sprattia venacavincola* (arrow) trapped in a glomerulus. H & E. (X400 magnification).

Plate 8.11: Section of tongue from *Trichosurus caninus* from Bulburin State Forest (central Queensland) showing a thick walled sarcocyst of a *Sarcocystis* sp. H & E. (X160 magnification)

Plate 8.12: Section of tongue from *Trichosurus caninus* from Bulburin State Forest (central Queensland) showing a thick-walled sarcocyst. Small zoites are visible inside the cyst, with fimbriae evident around the cyst wall. H & E. (X400 magnification).



CHAPTER 9

THE EFFICACY OF IVERMECTIN AND PRAZIQUANTEL FOR REDUCTION IN INTENSITY OF PARASITES INFECTING A WILD POPULATION OF MOUNTAIN BRUSHTAIL POSSUMS, *TRICHOSURUS CANINUS*, IN CENTRAL VICTORIA

9.1 INTRODUCTION

The impact of parasites on the demographics of wild vertebrate populations is largely unknown. Many parasitologists have explored the concept of population regulation by parasites and the stability of host-parasite interactions (Anderson and May 1978; May and Anderson 1978; Holmes 1982). However, there have been few studies of the effects of parasites on their hosts in the field (Gulland 1992; Hudson 1986; Bloomer *et al.* 1995), probably due to the logistical difficulties in collecting long-term demographic data in natural, free-ranging populations of host animals. Field-based experiments which involve the manipulation of parasite burdens may be used to test hypotheses about regulation of wild populations by parasites (Lehmann 1992; Krebs 1995; Sovell and Holmes 1996). In order to conduct such an experiment:- **(1)** the parasite fauna of the host species must be documented at the study site, and, **(2)** drugs which act effectively to reduce or remove those parasites which are most abundant and/or most pathogenic must be selected. For this study, a population of *T. caninus* at Cambarville in central Victoria was selected and preliminary studies of the parasite fauna in animals at that site were completed (Chapter 7). In this chapter, the efficacy is assessed of the antiparasitic drugs

ivermectin and praziquantel for removal of the known parasite fauna from *T. caninus* at Cambarville.

9.2 METHODS

Details of the study site at Cambarville in the central highlands of Victoria are given in Chapter 3.1.1. *T. caninus* was trapped during two week periods in January 1994 and April 1994. Faecal egg counts were completed for all animals (see 3.8.1).

9.2.1 Drugs

The anti-parasitic drugs ivermectin and praziquantel were selected for this experiment on the basis of information from previous post mortem surveys of the internal and external parasite fauna of *T. caninus* at Cambarville (see Chapters 7 and 8). The parasites which were recorded from the species at this site are given in Table 9.1. Ivermectin is a macrocyclic lactone. The mechanism of action of ivermectin is not fully understood but involves irreversible opening of chloride channels in parasite muscle membranes (Conder and Campbell 1995). This may affect the functioning of the pharynx, thereby affecting ingestion and excretion of nutrients by the parasite or its regulation of turgor pressure (Geary *et al* 1993). Ivermectin has broad spectrum activity against both the mature and immature stages of most parasitic nematodes, including gastrointestinal nematodes, lungworms and microfilariae (Campbell and Benz 1984; Conder and Campbell 1995). It also has efficacy against a wide range of arthropod ectoparasites, including ticks and mites (Lo *et al.* 1985; Conder and Campbell 1995). In addition, ivermectin may have persistent activity against some nematode species for 7-28 days after administration (Lo *et al.* 1985; Conder and Campbell 1995). These features of the drug were advantageous given the logistical constraints of field research which dictated an interval of eight to ten weeks between treatments.

Praziquantel has known activity against cestode parasites (Prescott 1986). This drug was selected to remove the cestode parasite, *Bertiella trichosuri* which has been recorded from *T. caninus* at Cambarville (see Chapter 7).

9.2.2 Post mortem examinations

Post mortem examinations (see Chapter 3.10) were completed post-treatment for three animals to test the efficacy of ivermectin and praziquantel for the removal of helminth parasites. The number of necropsy examinations completed was limited by difficulties in obtaining permits to euthanase more animals. One male animal (C94) was held for 48 hours after treatment with ivermectin and praziquantel, then sedated with tiletamine/zolazepam and euthanased by intracardiac injection of phenobarbitone, for post mortem examination. The other two animals (C101 and C103) were treated with the test drugs and then released at the point of capture. These animals were recaptured after ten days and detailed post mortem examinations were completed to examine for the presence of helminths and ectoparasites. Due to the small number of animals examined, statistical analyses were not completed and only descriptive results are presented. Ongoing demographic studies of *T. caninus* at this study site and difficulties in obtaining permits to remove animals due to fauna protection laws, precluded the removal of higher numbers of animals for post mortem examination.

9.2.3 Faecal examinations

Nine female *T. caninus* were sedated, weighed and injected with ivermectin (@ 200µg/kg) and praziquantel (@ 10mg/kg) by subcutaneous injection. Nine additional female *T. caninus* (control animals) were sedated, weighed and sham injected with sterile water. Animals were released at the point of capture.

To assess the immediate efficacy of ivermectin for reduction of egg counts in treated animals, faecal samples were collected from any of these animals that were re-captured during each two week trapping session. Pre- and post-treatment (within four days) faecal egg counts in treated animals (N=15) were examined for significant

difference using mixed models, as data were unbalanced and included within and between animal variation See 3.12.4).

For logistical reasons, it was beyond the scope of this study to determine the period of efficacy of ivermectin and praziquantel against the various target parasites. However, as part of an ongoing experiment (see Chapter 10), *T. caninus* (N = 25) were re-trapped eight weeks later, in April 1994. Faecal samples were collected from all animals and faecal egg counts were completed to determine whether those animals which were treated with ivermectin had been re-colonised with parasites after eight weeks. Given that the data were unbalanced and included both within and between animal variation in faecal egg counts, mixed models (see Chapter 3.12.4; restricted maximum likelihood (REML; Engel 1990)) were used to compare egg counts between treatment groups. In addition, variation was examined between faecal egg counts in treatment and control animals, eight weeks after treatment. This analysis was based on a single observation in April 1994, from treatment and control animals, so there were no repeat measures, and linear regression could be used (Chapter 3.12.2).

9.3 RESULTS

9.3.1 Post mortem results

At necropsy of animal C94, 48 hours after treatment with ivermectin and praziquantel, numerous *Paraustrostrongylus trichosuri* were present in the small intestine, colon and rectum. All parasites were beginning to uncoil and were no longer in contact with intestinal villi. Uncoiling *P. trichosuri* were most common in the contents of the small intestine but were evenly distributed at lower intensities throughout the contents of the large intestine and colon and in faeces within the rectum. *Bertiella trichosuri* was not detected.

T. caninus C101 and C103 were re-captured and necropsied ten days after treatment. No helminths were found at necropsy, and no eggs were found in faeces collected at post mortem. However, faecal egg counts performed on faeces collected

prior to treatment with ivermectin indicated that both of these animals had harboured intestinal nematodes that were shedding eggs before drug administration.

Ectoparasites were recovered from C94, C101 and C103 prior to initial treatment with ivermectin and praziquantel (see Table 9.2). Animal C94 was never released and therefore had no opportunity for re-infestation. Both C101 and C103 were not infested with ectoparasites when recaptured ten days after treatment with ivermectin, prior to necropsy.

9.3.2 Faecal examinations

Faecal egg counts collected from all *T. caninus* prior to treatment (N = 25), showed that all animals initially were infected with helminth parasites. Faecal egg counts in *T. caninus* do not necessarily reflect the intensity of infection with intestinal helminths (see Chapter 7), therefore quantification of the level of infection prior to treatment with ivermectin was not possible. Results of faecal samples collected from treated animals that were re-trapped within the two week trapping period (within four days of initial capture; N = 15) were not significantly different (Fig 9.1).

Faecal egg counts completed for animals that were re-trapped in April 1994 as part of an ongoing experiment (N=25; see Chapter 10) indicated that all animals had been recolonised with intestinal helminths during this time. Faecal egg counts in treated animals were not significantly different before, or eight weeks after, treatment (Fig 9.2). In addition, nematode egg numbers in treated animals were not significantly different from sham injected animals.

9.4 DISCUSSION

9.4.1 Efficacy of drug treatment

Observations on the efficacy were limited to necropsy examinations post-treatment of only three animals. The sample size for these tests was limited by difficulties in obtaining permits to euthanase more animals, given that *T. caninus* is a

protected species. Ivermectin appeared to effectively remove gastrointestinal helminths from *T. caninus* by ten days after treatment. Numerous *Paraustrostrongylus trichosuri,* but no *Parastrongyloides trichosuri* were present in the small intestine of C94 at post mortem, 48 hours after treatment. Those *Paraustrostrongylus trichosuri* that were present were beginning to uncoil and appeared to be dead or dying. *Paraustrostrongylus trichosuri* is usually found tightly coiled around the villi of the proximal small intestine (Mawson 1973; Smales and Mawson 1978; Presidente 1984). Uncoiling of these parasites in *T. caninus* treated with ivermectin was abnormal and indicates that they were being adversely affected by the drug.

In previous post mortem examinations of *T. caninus* from a number of sites across the host's distribution (see Chapter 7), *Paraustrostrongylus trichosuri* was detected at highest intensity in the duodenum, with lower numbers of parasites being present in the proximal ileum, beyond which it was not detected (pers. obs.). In contrast, *Paraustrostrongylus trichosuri* recorded in C94 were detected throughout the length of the small and large intestine. It appears that the dead and dying parasites were being moved along the gastro-intestinal tract away from their usual niche and excreted via peristalsis. Hence, although this necropsy was conducted only 48 hours after administration of ivermectin, it appeared that this parasite was being successfully removed.

Nematode eggs were detected in faecal samples that were collected from all *T. caninus* in this study prior to treatment, indicating that they initially harboured intestinal helminths. There was no significant decrease in faecal egg counts in animals that were treated and then re-captured within four days of treatment (see Fig 9.2). Faecal passage time may have influenced these results, however there is currently no information on retention or passage time for *T. caninus*. No intestinal helminths were recovered from either *T. caninus* C101 or C103 ten days after treatment with ivermectin and praziquantel. Thus it appeared that ivermectin began to remove intestinal nematodes in *T. caninus* from about 48 hours after treatment. This was not

reflected in faecal egg count four days after initial treatment. However, by ten days post-treatment in necropsied animals, all intestinal nematodes appeared to have been removed by treatment with ivermectin.

The efficacy of praziquantel for the removal of the cestode parasite *Bertiella trichosuri* is uncertain. Methods for detecting eggs of *B. trichosuri* in the faeces of *T. caninus* were unsuccessful (see Chapter 3.8.2), therefore it was not possible to determine the presence of this parasite prior to treatment with praziquantel. *B. trichosuri* was not detected in treated *T. caninus* at post mortem. However, it was not possible to determine whether these animals were uninfected with *B. trichosuri* prior to treatment, or, if any *B. trichosuri* present were removed by treatment with praziquantel. Post mortem examinations of other animals from this region have revealed a 63% prevalence of this parasite (see Chapter 7). Despite this, the evidence of the efficacy of praziquantel for removal of *B. trichosuri* was not conclusive.

It was difficult to determine the efficacy of ivermectin for removal of ectoparasites. Ectoparasites were collected from all three animals targeted for euthanasia at the time of initial treatment. *T. caninus* C94 was held for 48 hours prior to euthanasia and had no opportunity for re-infestation, hence the efficacy of ivermectin for the prevention of re-infestation with ectoparasites could not be determined in this animal. At the time of treatment, *T. caninus* C101 and C103 were carrying moderate burdens of ectoparasites (see Table 9.2). These animals were released at their point of capture. At necropsy ten days later, these animals were not infested with ectoparasites. The period of efficacy of ivermectin against arthropod ectoparasites is unknown. In cattle, mites are initially found for five days after treatment, after which time they rapidly disappear (Campbell and Benz 1984). Studies on cattle have also shown that although ivermectin does not necessarily result in immediate death or detachment of ticks, it does appear to interfere with various biological processes that are essential to tick survival and reproduction (Campbell and Benz 1984).

9.4.2 Recolonisation of hosts by helminth parasites

Several host factors may contribute to the likelihood of infection and recolonisation of hosts by helminth infective stages, such as differences in habitat, age, immunocompetence, home range size, activity, and diet (Keymer and Anderson 1979; Munger and Karasov 1991; Haukisalmi *et al.* 1995). Helminth parasites generally exhibit patterns of overdispersion in the host population; some hosts being heavily infected, whilst most others carry relatively low burdens of parasites (Bradley 1972; Anderson and May 1978). Given this, infective stages of parasites are also likely to be unevenly distributed, ie. there will be foci of infection, mostly within the ranges of those animals which are more heavily infected (Crofton 1971; Bradley 1972; Anderson 1978; Keymer and Anderson 1979; Holmes and Price 1986). In *T. caninus* at Cambarville, infective stages of parasites are probably reacquired at a similar rate before and after treatment, given that animals were released at the point of capture within their known home ranges. The apparent re-colonisation of *T. caninus* with intestinal helminths within eight weeks indicates a fairly rapid parasite acquisition rate. Alternatively, ivermectin may not have been effective for the removal of immature larvae that were dormant or migrating at the time of treatment (see Sovell and Holmes 1996). Such larvae may have subsequently matured and commenced shedding eggs in the faeces .

The foraging behaviour of *T. caninus* probably influences the rate of re-colonisation with some parasite species. *T. caninus* is omnivorous and forages for food that occurs in both the understorey vegetation and on the ground (Chapter 2.6). Foraging on the ground may expose *T. caninus* to ingestion of the infective stages of intestinal helminth species, as well as oribatid mites, which may serve as intermediate hosts for *Bertiella trichosuri* (Gleason and Buckner 1979; Viggers and Spratt 1995). Oribatid mites have been recovered from the pelage of *T. caninus* at Cambarville, and may be ingested by *T. caninus* during grooming. However, no developing cysticercoids were observed in these mites (pers. obs.).

All treated *T. caninus* were shedding eggs in their faeces when they were re-trapped eight weeks after treatment with ivermectin and praziquantel. Sovell and Holmes (1996) found that treatment with ivermectin reduced the number of nematodes in free-ranging snowshoe hares, *Lepus americanus*, in the Yukon, USA, for only four weeks. They suggested two possible reasons for this:- **(1)** ivermectin may not prevent re-infection with parasites, even during peak drug activity, and/or, **(2)** treatment with ivermectin did not kill immature or dormant larvae in the tissues of the host. The period of efficacy of ivermectin in *T. caninus* was not determined in this experiment.

Sovell and Holmes (1996) suggested that frequent re-treatment (ie. every two to four weeks) would be required in *L. americanus* to maintain low parasite burdens, given that treatment with ivermectin did not appear to have long-lasting effects. The required interval for re-treatment of *T. caninus* to maintain faecal eggs counts at a lower level in treated animals than in untreated individuals is unknown, but clearly is less that eight weeks. Sovell and Holmes (1996) suggested that the need for frequent re-treatments of animals may be reduced by the use of a sustained-release bolus or implantable capsule (Alva-Valdes *et al.* 1988). This approach may be considered for future field experiments of this nature.

The time for re-colonisation of *T. caninus* with ectoparasites was not determined in this study. Sources of re-colonisation of *T. caninus* with ectoparasites may include the surrounding vegetation in which the animal forages and nest sites in hollow-bearing trees (Lindenmayer *et al.* 1994c). The level of infestation of nest sites with ectoparasites may influence the rate of recolonisation of *T. caninus* with these parasites after treatment with ivermectin. Lindenmayer *et al.* (1996a) found that *T. caninus* at Cambarville frequently changed nest sites. Den swapping behaviour may partly be associated with ectoparasite avoidance (Lindenmayer *et al.* 1994c).

9.6 CONCLUSIONS

Although the results from necropsy data presented here were limited because they were based on findings from only three animals, it appeared that ivermectin successfully removed intestinal nematodes from *T. caninus* between two and ten days after treatment. Faecal egg counts did not decrease within four days of treatment with ivermectin and praziquantel, and treated animals were re-colonised with parasites and shedding eggs by eight weeks after treatment. Treatment with ivermectin reduced ectoparasite burdens in two animals necropsied ten days after treatment. However, the efficacy of this drug for the prevention of reinfestation of *T. caninus* with ectoparasites is unknown. Findings for the efficacy of praziquantel against *B. trichosuri* were inconclusive.

The period of efficacy of ivermectin and the rate of recolonisation of *T. caninus* with various helminth and ectoparasites was not determined in this field trial. Under ideal circumstances, the re-treatment interval should be based on knowledge of parasite pre-patent periods and the period of efficacy of the drugs against the known parasite fauna.

Although findings for this field trial were largely descriptive due to small sample sizes, the drugs ivermectin and praziquantel were considered likely to be effective for the removal, or reduction in burdens of the helminths, and at least some of the ectoparasites, known to occur in *T. caninus* at Cambarville. On this basis, these drugs were used in the field experiment described in Chapter 10.

Table 9.1: The parasites recorded from *T. caninus* at Cambarville

Endoparasites		**Location**
Nematodes	*Paraustrostrongylus trichosuri*	proximal small intestine
	Parastrongyloides trichosuri	proximal small intestine
Cestodes	*Bertiella trichosuri*	distal small intestine
Ectoparasites		
Mites	*Trichosurolaelaps trichosuri*	
	T. dixous	
	Haemolaelaps penelope	
	H. sisyphus	
	Atellana papilio	
	Cheyletiella parasitivorax	
Ticks	*Ixodes tasmani*	
	I. trichosuri	
Fleas	*Acanthopsylla rothschildi rothschildi*	

Table 9.2: Arthropod ectoparasites collected from three *T. caninus* prior to treatment with ivermectin and praziquantel

Animal	Ectoparasite species	Intensity
C94	*T. crassipes*	20
	T. dixous	3
	A. papilio	2
	P. dycei	6
C101	*I. tasmani*	1 adult
	I. trichosuri	1 adult
	A.r. rothschildi	1
	T. crassipes	8
	T. dixous	4
	H. penelope	5
	A. papilio	4
	P. dycei	6
C103	*T. crassipes*	30
	T. dixous	6
	H. penelope	21
	A. papilio	40
	P. dycei	59

Fig 9.1: Faecal egg counts from *T. caninus* (N=15) before and four days after treatment with ivermectin (means and approximate 95% confidence intervals are shown)

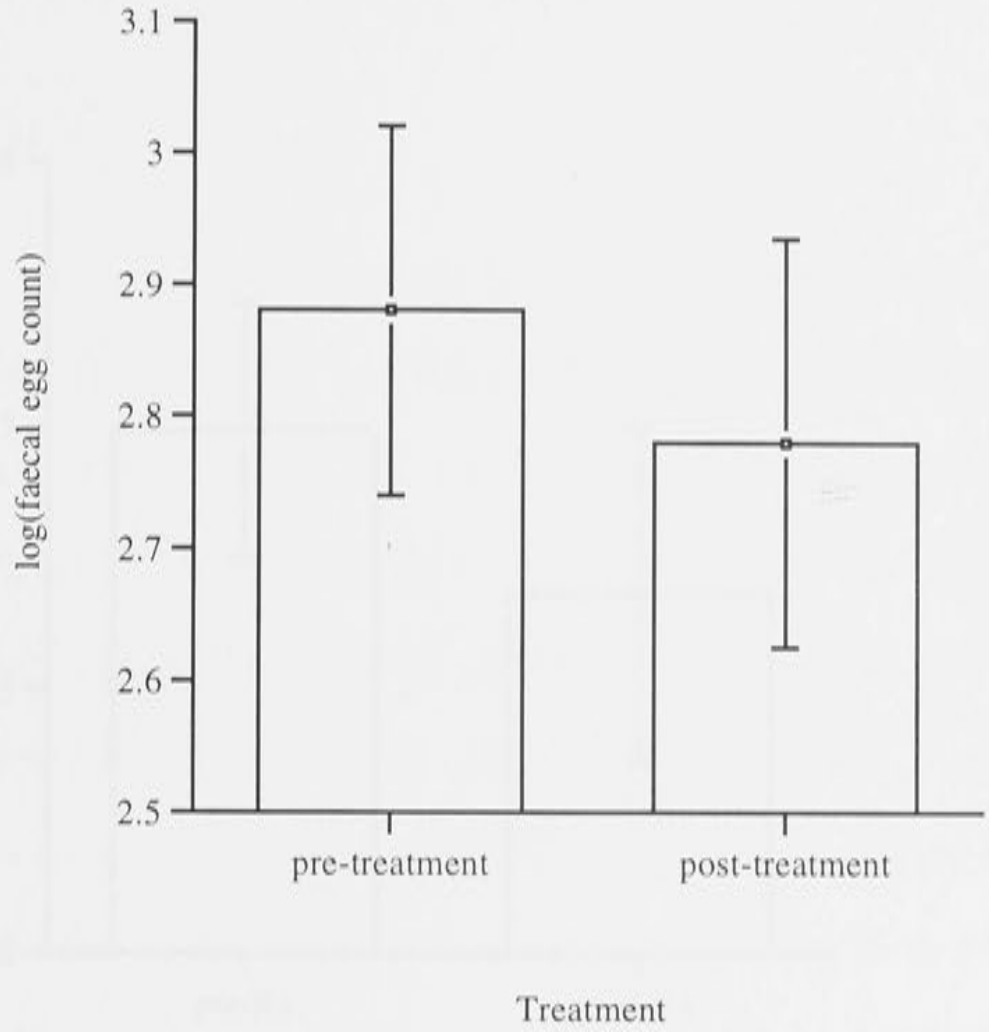
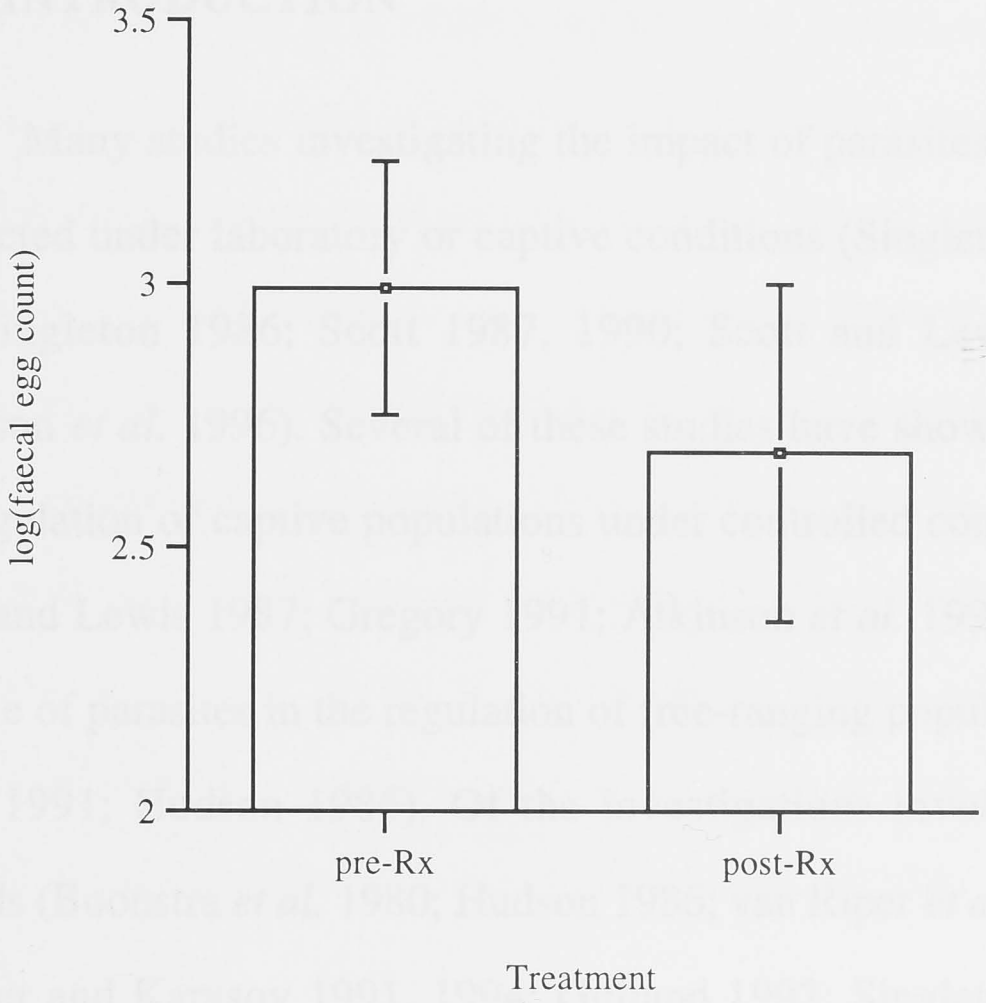


Fig 9.2: Faecal egg counts before and eight weeks after treatment with ivermectin in *T. caninus* (N=25) at Cambarville (means and approximate 95% confidence intervals are shown)

p = 0.51



Label: Rx = treatment

CHAPTER 10

THE EFFECT OF REMOVAL OF PARASITES ON THE HEALTH AND REPRODUCTIVE SUCCESS OF ADULT FEMALE MOUNTAIN BRUSHTAIL POSSUMS, *TRICHOSURUS CANINUS*, IN CENTRAL VICTORIA.

10.1 INTRODUCTION

Many studies investigating the impact of parasites on populations have been conducted under laboratory or captive conditions (Singleton and Spratt 1986; Spratt and Singleton 1986; Scott 1987, 1990; Scott and Lewis 1987; Gregory 1991; Atkinson *et al.* 1996). Several of these studies have shown an effect of parasites on the regulation of captive populations under controlled conditions (Scott 1987, 1990; Scott and Lewis 1987; Gregory 1991; Atkinson *et al.* 1996). However, evidence for the role of parasites in the regulation of free-ranging populations is limited (Boonstra *et al.* 1991; Hudson 1986). Of the investigations involving wild populations of animals (Boonstra *et al.* 1980; Hudson 1986; van Riper *et al.* 1986; Barker *et al.* 1991; Munger and Karasov 1991, 1994; Gulland 1992; Singleton *et al.* 1995; Sovell and Holmes 1996), there have been only a few attempts to manipulate parasite burdens in the field to study the role of parasites in limiting and regulating wild populations (Hudson 1986; Hudson *et al.* 1992; Gulland 1992; Lehmann 1992; Singleton and Chambers 1996).

The regulation of natural populations may be influenced by a number of factors including parasites and disease, nutrition, predation, territoriality and the environment (Holmes, 1982, 1995; Krebs 1995). The potential impact of any of these individual factors needs to be considered in the context of the other pressures on populations (Holmes 1982; Anderson 1996). Parasitism and disease may contribute to the loss of

host fitness by lowering probability of survival, impairing growth rates, reducing access to mates and reducing fertility (Boonstra *et al.* 1980; Freeland 1986; Hudson 1986). There are differing views on the role of parasites and diseases in the regulation of natural populations. Some authors suggest that there is evidence for host population regulation by parasites (Anderson and May 1978, 1979; May and Anderson 1978, 1979), while others propose that, although diseases and parasites may contribute to host mortalities, these deaths are compensatory (ie. occur in place of other forms of mortality), and therefore do not play a role in population regulation (Holmes 1982).

Anderson and May (1978) and May and Anderson (1978) used mathematical models to describe a number of factors that influence host-parasite interactions and the potential for population regulation by parasites. They demonstrated that parasites must influence host birth, death or movement rates in order to regulate host populations. The patterns of dispersion of parasites within host populations also may affect host-parasite interactions (Anderson and May 1978). In general, parasites are overdispersed due to host variation in immunological status and exposure to infection (Bradley 1972; Anderson and May 1978). If parasites are highly aggregated within the host population, they may not have a regulatory effect, as they may be eliminated with the death of a small number of heavily infected hosts (Bradley 1972; Anderson 1978).

Parasite populations within vertebrate hosts are generally considered to be subject to density dependent constraints resulting from host immunological responses and intraspecific competition between parasites for host resources (Anderson and May 1978). Highly pathogenic parasites may either kill the host rapidly or induce a large host immunological response to reduce parasite numbers or fecundity, and are therefore unlikely to regulate host populations (Bradley 1972; Anderson 1979; Freeland 1986; McCallum 1994). Similarly, parasites of extremely low virulence are unlikely to influence host population density, as they will have minimal effect on the fitness of the host (Anderson 1979; Freeland 1986; McCallum 1994).

Mathematical models are useful for exploring the theoretical concepts of population regulation by parasites and may be used to guide and interpret field research (McCallum 1995; Singleton *et al.* 1995). However, field-based studies and experiments are essential to further explore the role of parasites in the regulation of natural populations and, in turn provide, the data to improve theoretical models (Krebs 1995; McCallum 1995). The effects of disease and parasites on free-ranging populations may be difficult to detect and quantify, as those animals that are debilitated may be rapidly removed by predators and scavengers, or animals may die in inaccessible locations (McCallum and Dobson 1995). Given this, there are two main methods whereby field data on the effects of parasites on host populations may be obtained:- (**1**) collection of data on the disease status of animals within the context of a mark-recapture experiment (*sensu* Pollock *et al.* 1990; Lebreton *et al.* 1992) to look for associations between disease and survival or fecundity (Boonstra *et al.* 1980; McCallum and Dobson 1995), or, (**2**) manipulation of disease or parasite burdens in part of the population, to compare survival and fecundity with control animals within the same population (Krebs 1995; McCallum 1995; McCallum and Dobson 1995; Bloomer *et al.* 1995). The latter approach allows hypotheses about population regulation to be tested in the field. Studies which compare naturally infected and uninfected hosts are not appropriate, as they lack suitable controls and are confounded by the presence of the factors which caused the original difference in level of infection between hosts (Munger and Karasov 1991).

In this chapter, a field-based parasite manipulation experiment is described for *T. caninus* at a single study site at Cambarville in the central highlands of Victoria. The aims of this experiment were to:- (**1**) determine whether treatment to remove or reduce parasite burdens in adult female *T. caninus* had an effect on the birth rate, survival or growth rate of dependent young, (**2**) establish whether it was likely that parasites were having a regulatory effect on this population of *T. caninus*, and, (**3**) examine the effects of reduction of parasite burdens on the haematological and serum biochemical

values of adult female *T. caninus*. The implications of the findings of this experiment are discussed.

10.2 METHODS

10.2.1 Background information

Information gathered from the study by How (1972) of *T. caninus* at Clouds Creek demonstrated a high loss of dependent young, despite considerable maternal investment. Only 36% of young born survived to emergence from the pouch (How 1972). There was no mortality of young during the progression from pouch young stage to back young stage (How 1972), which would be a period of maximum vulnerability to predation. Therefore, most mortality of young occurred during pouch life. In the first two years of the current project studying *T. caninus* at Cambarville, a high loss of young prior to emergence from the pouch also was recorded (Table 10.1).

The parasite fauna of *T. caninus* at Cambarville was documented as part of a study of the species across its geographic range (see Chapters 7 and 8). An experiment to test the efficacy of the drugs ivermectin and praziquantel for the removal of those parasites also has been described (see Chapter 9). On the basis of this information, an experiment was completed to manipulate parasite burdens in a population of free-ranging *T. caninus* to observe the effects of a reduction of parasite burdens on a range of factors.

10.2.2 Trapping and handling procedures

Details of the study site at Cambarville have been outlined in Chapter 3.1.1. Female *T. caninus* were trapped during 10 day periods, at 8-10 week intervals from January 1994 to November 1994 (see Table 10.2). For each animal in each trapping period, a blood sample was collected for haematological and serum biochemical analyses (see Chapter 3.5 and 3.6), a range of morphological measurements were recorded, including body mass and age-class (see Fig 3.2), and reproductive status

was determined. Ectoparasites were not collected from females carrying dependent young, due to the risk of killing the young. However, a sample of faecal pellets was collected for subsequent faecal egg counts (see Chapter 3.8.1). Pouch young, when present, were weighed only if detached from the teat and several morphometric measurements were recorded, including head length, crown-rump length, body length and tail length. The sex of each young also was noted. Given that the exact birth date of each young was not known, the age of young at first capture was estimated on the basis of size in relation to the commencement of the breeding season.

In January 1994, a considerable trapping effort (60 traps x 14 nights) was undertaken in an attempt to capture all adult female *T. caninus* within the Cambarville study site. Animals were assigned to treatment (N=9) and control groups (N=8) (see Table 10.2). After April 1994, any new females that were captured were added to the control group (N=3). Animals in the treatment group were injected subcutaneously with ivermectin (200µg/kg) and praziquantel (10mg/kg) using a separate syringe for each drug. Sham injections of sterile water were administered to those animals assigned to the control group. All animals were re-treated (drug or sham injected) at 8 - 10 week intervals from January 1994 - November 1994 (see Table 10.2). Reproductive success was determined by survival of young to emergence from the pouch. Emergence was defined as survival to November 1994, when all surviving young would have emerged from the pouch to enter the back young phase or semi-independence, whilst continuing to suckle from the mother. Any young that disappeared prior to November 1994 would still have been dependent, and therefore were assumed to have died.

10.2.3 Statistical analyses

10.2.3.1 Growth rate of young

The effect of removal of parasites from adult female *T. caninus* on the growth rate of dependent young was examined. Only pouch young which were captured with

their mother on more than one occasion were included in these analyses. First, the relationship between the two continuous variables "Age" of the young (days) and its head length (mm) was examined using linear regression (see Chapter 3.12.2; Weisberg 1980). A regression line from repeat morphometric measures during the pouch life of the young was obtained for each young. For each individual, the slope of the regression line was calculated to form a new variable; "growth rate", which had a single level. Variation in the growth rates of young was then examined using linear regression to determine whether there was an effect of treatment of the mother on the growth of young. In addition, a number of variables derived from the mother were examined for significant effects on the growth rate of young. These variables included body mass and age-class, as well as a range of haematological and serum biochemical values (Table 10.3). Given that repeat measures for variables from mothers captured more than once throughout the experiment could not be incorporated into the statistical model with a single level response variable (growth rate), the mean and the range of each variable were calculated and used in the modelling process.

10.2.3.2 Birth rate and probability of survival of young

The effect of removal of parasites from mothers on the birth rate (proportion of births) and probability of emergence of dependent young also was examined. Given that the response variable (the probability of emergence) had a binary outcome (1,0; ie. emergence, non-emergence), logistic regression analysis (see Chapter 3.10.3; Collett 1991) was used to examine the effects of a range of variables on the survival of young. The potential explanatory variables included the sex of the young and blood variables measured from the mother (Table 10.3), as well as the mother's body mass and age-class.

10.2.3.3 Haematological and serum biochemical values of adult female T. caninus

Haematological and serum biochemical values for adult female *T. caninus* were examined for significant differences between treatment and control groups. Data for all

females from all trapping sessions were combined for the analyses. As not all females were trapped in every trapping session, data were unbalanced, so mixed models (see 3.12.5) were used to take account of between animal and within animal variability.

10.3 RESULTS

T. caninus were captured during five trapping sessions between January 1994 and November 1994 (Table 10.2). In January 1994, 17 females were trapped and assigned to treatment and control groups (Table 10.2). In April 1994, an additional female (C43), was added to the treatment group, and a previously trapped treatment female (C50) was not captured. In June 1994, all control females were trapped, but animal C43 was missing from the treatment group. An aged treatment female, C15, was not re-trapped after April 1994. In August 1994, C50 and C32 were not trapped from the treatment group, and in November C32, C50 and C28 (control) were not trapped.

Data on the survival of young between trapping periods for treatment and control groups are given in Table 10.4. Early in the season (April 1994) 66% of females in the treatment group had given birth to young, compared with 25% in the control group of females (see Table 10.5). By June 1994, all females in the treatment group and 88% of females in the control group were carrying pouch young. There was loss of one young from the control group by August 1994. No mortality of young occurred in the treatment group during this time. In November 1994, 69% of young recorded in the treatment group were still alive, compared with 60% in the control group.

The proportion of females successfully giving birth to young was higher in the treatment group than in the control group (Table 10.5). This difference was due to one female in the control group (C21) not giving birth. The proportion of females in the treatment group that gave birth did not differ from population data collected at Cambarville over the preceding two years (see Table 10.1). The proportion of females

that successfully reared young to emergence from the pouch was 0.69 for females treated with ivermectin and praziquantel, and 0.60 for control animals (Table 10.5). This difference was not significant. Therefore, there was no evidence from these data that the treatment of adult female *T. caninus* with ivermectin and praziquantel affected the probability of survival of young to emergence from the pouch. However, sample sizes were small and the power of the experiment was subsequently low.

The effects of treatment of adult female *T. caninus* with ivermectin and praziquantel on the growth patterns of dependent young were examined. First, growth curves for each young were derived. The relationship between age and head length of pouch young is shown in Fig 10.1. The slope of each line, derived by linear regression analysis, represented the growth rate of each individual pouch young. Linear regression was then used to determine whether treatment of the mother to reduce parasites affected the growth rate of pouch young. No effect of treatment was found. In the initial full model that was tested and throughout the repetitive modelling process, the variable "treatment" had the least effect of any variables on the response variable "growth rate", and was the first variable to be dropped on each occasion. This indicates that the effects of treatment were extremely minimal and certainly not significant. There was no significant effect of any maternal variables on the growth rates of young.

Logistic regression analysis was used to examine the significance of potential explanatory variables on the survival of pouch young. Variables tested included the sex of the young, and a number of maternal variables. No effect of treatment of mothers was found on the survival of young to emergence from the pouch. The variable "treatment" was one of the first to be dropped from the modelling process due to its lack of significance.

An effect of treatment to reduce parasite burdens on the haematological and serum biochemical values of adult female *T. caninus* was detected only for the absolute eosinophil count. Counts were lower in treated animals in autumn than in the

control animals (Fig 10.2). There was no significant difference in eosinophil count between treated and untreated animals in any other season.

10.4 DISCUSSION

Animals that are ill are rarely detected in free-ranging populations, hence it is often difficult to establish associations between the presence of a disease agent or parasite, and ill health or mortality in the host (McCallum 1995). Given that parasites may exert effects that are not clinically apparent on their hosts (Freeland 1986), a field-based experiment was completed in this thesis to investigate the effect of reduction of parasite burdens in adult female *T. caninus* on haematological and serum biochemical values, as well as on the survival and growth rates of dependent young.

Early in the breeding season (April 1994), more females in the treatment group (66%) had given birth to young compared with the control group (25%), however, this difference was not significantly different. The first treatment to remove parasites was completed in January 1994, prior to the onset of the breeding season. It is possible that reduction in parasite burdens may have contributed to the higher early conception rates in the treatment group of females.

By June 1994, all female *T. caninus* treated with ivermectin and praziquantel had given birth to young, compared with 88% in the control group. The population of *T. caninus* at Cambarville appears to be highly fecund and most adult females give birth to young every year. However, not all females carry their young successfully through to emergence from the pouch (see Table 10.1). In the study by How (1972) at Clouds Creek, the fecundity of adult female *T. caninus* decreased gradually with age (see Table 10.7). No age-specific variation or decline in fecundity was observed in *T. caninus* at Cambarville.

There was no significant detectable effect of treatment of *T. caninus* with ivermectin and praziquantel on the survival or growth rates of dependent young. A total of 69% of females in the treatment group successfully reared young to emergence

from the pouch, compared to 60% in the control group. However, the sample sizes in this experiment were small, so the likelihood of detecting small differences in survival or growth rates was low. The proportion of surviving young in the year of the experiment was higher than in 1992. However, in the year following the experiment (1995), an even higher proportion of young survived to emergence (80%). This difference is unlikely to be due to acclimatisation to trapping, as in each year of the study new animals were captured on the trapping grid, whilst others disappeared from the study area. Therefore, the group of trapped animals was in a state of flux which would negate acclimatisation effects. The variation between years in data on survival of young of *T. caninus* emphasises the value of long-term field studies to reduce the year to year variability which may mask trends in short term data.

There are many factors which may have contributed to birth rate and survival of *T. caninus*, such as climate, nutrition, predation and social status. Survival of young of *T. caninus* at Cambarville increased in each year of the study from 1992. This may have been due to improved climatic conditions after a drought in 1992, which may have facilitated better plant growth and improved nutrition, resulting in a higher success rate of survival of young.

Numerous studies on captive or laboratory animals have reported effects of parasitism of hosts (Scott 1987, 1990; Scott and Lewis 1987; Gregory 1991; Atkinson *et al.* 1996). However, only findings from studies on free-ranging populations will be discussed here. A low energy intake, in conjunction with a high parasite burden may negatively affect survival of animals in wild populations (Gulland 1992; Holmes 1995). In a naturally regulated population of Soay sheep, *Ovis aries* L., on St Kilda Island in the Outer Hebrides, a periodic density dependent die-off occurs, with up to 75% mortality of animals (Gulland and Fox 1992). Gulland (1992) completed a field experiment to examine the effects of removal of parasite burdens on the population crash. Necropsied animals were generally emaciated and carried high nematode burdens. Removal of parasites initially increased daily survival rate, but did not

prevent re-infection as the population crash progressed (Gulland 1992). It appeared that parasites were not regulatory in *O. aries*, but probably contributed to mortality in hosts already suffering from protein-energy malnutrition and exacerbated the effects of food-shortage (Gulland 1992). A similar finding was reported by Arundel *et al.* (1990) in a study of mortality in juvenile eastern grey kangaroos, *Macropus giganteus*, in which mortality was attributed to a combination of parasitism and poor nutrition. Given the potentially major role of nutrition in survival and fecundity, and its possible interaction with the effects of parasites, this factor must be considered in the context of the field experiment in *T. caninus* at Cambarville. It is possible that the year during the field experiment was conducted (1994) and the following year (1995), may have been years of particularly high food availability for *T. caninus* at Cambarville. This may have enhanced the survival of young. In other years, during which animals have been captured with similar frequency, the survival of young was considerably lower.

T. caninus at Cambarville that were treated to remove parasites were released back to their home range, in which continuous reinfection with parasites was possible. All animals were reinfected with parasites within eight weeks of treatment (see Chapter 9), and there was no significant difference between faecal egg counts in animals treated with these drugs and control animals at the commencement of a new trapping period eight weeks later (Chapter 9). It is possible that pathogenic effects associated with re-colonisation by parasites may have negated any benefits of treatment to remove parasites. The problem of reinfection with parasites could be partially overcome by treating animals more frequently (see Chapter 9; Sovell and Holmes 1996). The experimental design whereby animals are captured and re-released back into a contaminated environment would not be accepted in studies of domestic animals such as sheep (see Morely and Donald 1980). However, in studies of field populations of wild mammals, elaborate experiments with each treatment group replicated and held in separate paddocks or enclosures (see Morley and Donald 1980) are not logistically possible.

A number of studies have reported a lack of detectable effect of parasites on population dynamics of free-ranging populations (Munger and Karasov 1991; Bloomer *et al.* 1995; Singleton and Chambers 1996). In a study of a non-cyclic population of the snowshoe hare, *Lepus americanus*, in central Wisconsin in the USA, Bloomer *et al.* (1995) showed that although ivermectin markedly reduced the prevalence and intensity of nematodes infecting *L. americanus*, there was no effect of treatment on body condition, reproduction, survival, home range sizes or susceptibility to predation. *L. americanus* appeared to develop immunity to the nematode parasites which infected them, and this may have contributed to the comparatively low worm burdens detected in this species (Keith *et al.* 1986). It is possible that immunity to internal parasites in *T. caninus* at Cambarville may have stabilised parasite burdens at low levels which were unlikely to have a significant pathogenic or regulatory effect on the species.

In contrast to the findings for *T. caninus* at Cambarville, some studies on other animals have detected significant negative effects of parasitism on host survival, reproduction and growth rates. Studies on the red grouse, *Lagopus lagopus scoticus*, in northern England have shown a significant effect of the caecal nematode, *Trichostrongylus tenuis,* on host clutch size and survival (Hudson 1986; Hudson *et al.* 1992). Female *L.l. scoticus* that had been treated with an anthelmintic to reduce *T. tenuis* infections, produced larger clutches than control birds, and the relative survival of treated birds over winter was higher in birds with reduced worm burdens (Hudson 1986; Hudson *et al.* 1992). Both of these effects were associated with the intensity of parasite infection in breeding grouse; effects on the host were more pronounced at higher intensities of infection with *T. tenuis* (Hudson 1986; Hudson *et al.* 1992).

Many studies have examined the effect of ectoparasites on the breeding success and nesting behaviour of birds (Møller 1990, 1993; Brown and Brown 1986, 1991; Chapman and George 1991; Lope *et al.* 1993; Richner *et al.* 1993). However, there have been only a few studies of a similar nature on mammalian hosts (Munger and

Karasov 1991; Lehmann 1992). Munger and Karasov (1991) conducted a field experiment to examine the effects of larvae of the botfly *Cuterebra angustifrons,* on the population density and regulation of white-footed mice, *Peromyscus leucopus* at a wildlife reserve in Wisconsin, USA. They showed that larvae of *C. angustifrons* had no effect on survival or reproduction. However, in a study of the impact of botfly *(Cuterebra grisea)* infestation on populations of voles, *Microtus townsendii*, parasitism was shown to negatively affect host survival, fertility and growth rates (Boonstra *et al.* 1980). Lehmann (1992) demonstrated that manual removal of ectoparasites (fleas, mites, ticks and lice) from field populations of gerbils, *Gerbillus andersoni allenbyi*, affected both host survival time and red blood cell parameters, although there was no effect on host body condition. In this study of removal of parasites from *T. caninus*, no effect of either nematodes or ectoparasites on the survival or growth rates of pouch young was detected.

Most haematological and serum biochemical values of adult female *T. caninus* were not affected by treatment with ivermectin and praziquantel to reduce helminths and ectoparasite burdens. Absolute eosinophil counts were significantly higher in treated females in autumn than females in the control group. There were no significant differences between eosinophil counts in any other season. In sheep, *Ovis ovis*, Stear *et al.* (1995) demonstrated that, after anthelmintic treatment, peripheral eosinophil counts rose rapidly after reinfection with *Ostertagia circumcincta*. Counts peaked after 7-14 days, until about six weeks after infection, when they fell to normal levels. Higher autumn eosinophil counts in *T. caninus* treated with ivermectin and praziquantel, may reflect a response to higher rates of parasitic re-invasion at this time. No other significant effects of treatment to reduce parasite burdens were found for blood values of *T. caninus*. However, it is possible that parasites may have pathogenic effects on this host in the presence of other disease. For example, in a study of the European rabbit, *Oryctolagus cuniculus*, in Scotland, Boag (1988) suggested that the viral disease myxomatosis reduced the immune response of infected rabbits, resulting in an increase in helminth infection.

10.4.1 Limitations of this study

Experiments to test the effects of removal of parasites in wild populations of mammals have been only rarely attempted. Given the extensive literature examining the possible effects of parasites on population regulation from a theoretical perspective, more field trials are required to test these theories. Such field experiments are difficult to undertake and labour intensive. It may be difficult to capture sufficient animals in order to detect suble differences in reproductive success (see McCallum 1995). For most species, an experiment to capture and treat hundreds of animals at regular intervals would not be logistically possible. However, smaller scale experiments can provide valuable information if there are substantial differences between control and treatment groups, and also provide a foundation for designing future experiments.

For domestic animals, a more ideally designed experiment would be possible. For example, in this study of T. caninus, the release of treated animals into a contaminated environment in which reinfection may have occurred rapidly may have interfered with the detection of treatment effects (see Morley and Donald 1980). In comparison, for domestic animals, a replicated experiment could be conducted with treated animals and control animals held in separate paddocks to eliminate this type of confounding effect (Morley and Donald 1980). However, an experimental design involving this type of manipulation of the environment would be extremely difficult when working with free ranging populations of mammals, and was not feasible for studies of *T. caninus*.

Treatment of adult female *T. caninus* to reduce parasite burdens did not appear to affect the birth rates, survival or growth rates of dependent young or most of the haematological and serum biochemical values of the host. There are several possible explanations for the lack of measurable effect of parasites on the demographic factors examined in this experiment. The sample size in this experiment may have been too small. McCallum (1994) and McCallum and Dobson (1995) have outlined some of the

difficulties in selecting sample sizes to quantify the effects of parasites on free-ranging populations. The site at Cambarville was selected for this study because *T. caninus* was known to occur at a relatively high density: 1.1 animals/ha (Lindenmayer unpublished data), compared with 0.4/ha in How's (1972) study of *T. caninus* at Clouds Creek. *T. caninus* was also known to be re-trappable at this site (Lindenmayer *et al.* 1996a, 1996b), which was important for trap-recapture studies and re-treatment of individuals. Considerable trapping effort was required to capture the animals included in this experiment, and it was unlikely that further trapping effort would have yielded many more animals. In addition, the unforseen disappearance of some of the older animals from the trapping grid lowered the number of animals, which may have reduced the likelihood of detecting a difference between the treatment and control groups. Two of the animals that disappeared had been reliably trapped in the same home range over the preceding four years and it was therefore assumed that they had died. Neither female had bred for three years. Interestingly, both females (C15 and C32) successfully gave birth to young after treatment with ivermectin and praziquantel. However, this association may not be causative and another factor, such as nutrition, may have been responsible for the successful births in the year of the experiment.

It appears likely that the parasites infecting *T. caninus* at Cambarville are of low pathogenicity. McCallum (1994) used mathematical models to demonstrate that parasites with low pathogenicity will only have a major effect on their hosts if density dependent constraints on parasites are weak. In general, vertebrate hosts are able to mount an immune response to helminth infection which decreases egg production and parasite survival as parasite burden increases (Anderson and May 1991). Given that these density dependent constraints are likely in vertebrate hosts, McCallum (1994) showed that parasites of intermediate pathogenicity will have the greatest effect on host population dynamics. Data from Chapters 7 and 8 and the findings from the experiment in this Chapter, suggest that the parasites which commonly infect *T.*

caninus at Cambarville are probably of low pathogenicity, and hence unlikely to exert regulatory effects on the host population.

The number of helminth species infecting *T. caninus* at seven study sites was relatively low (see Chapter 7). Parasite species richness at Cambarville was slightly lower than that occurring in host populations in other parts of the geographic range of the species (see Chapter 7). In particular, potentially pathogenic species such as *Marsupostrongylus minesi* and *Sprattia venacavincola*, were absent from the Cambarville population. The low diversity of parasite species infecting *T. caninus* at this site may have limited the potential for regulation of this host population by parasites.

10.5 CONCLUSIONS

The reduction of parasite burdens in adult female *T. caninus* at Cambarville in central Victoria had no detectable effect on the birth rate, survival or growth rate of dependent young, or on most of the haematological and serum biochemical values of the mothers. The findings from this study may differ if a larger scale experiment was completed (ie. using greater numbers of hosts). However, another suitable location for study of *T. caninus* with a higher density of animals, or where more animals could be trapped, is currently unknown. Equally, different results may be recorded at another study site where a greater diversity of helminth species was present, or even in another year. Alternatively, it may be useful to repeat this type of experiment using a different host species harbouring a more diverse helminth fauna.

Table 10.1: Reproductive success of adult female *T. caninus* at Cambarville in 1992, 1993 and 1995, assessed by proportion of females breeding, and proportion of young surviving to emergence from the pouch.

Year	Proportion giving birth	Proportion successfully rearing young to emergence
1992	1 (12/12)	0.3 (3/10)
1993	0.93 (14/15)	0.61 (8/13)[a]
1995	1 (13/13)	0.8 (12/15)[b]

[a] not all females that had pouch young early in the season were re-captured at the time of emergence of young

[b] more females were captured at the time of emergence of young than were trapped earlier in the season

Table 10.2: Identification numbers of female *T. caninus* in treatment and control groups that were captured in each trapping session, during an experiment to examine the effects of treatment of adult females with ivermectin and praziquantel to reduce parasite burdens, on the growth and survival of dependent young.

Trapping period	**Identity of females trapped**	
	Treatment	Control
20 Dec '93 - 2 Jan '94	15	5
	23	10
	24	28
	27	34
	31	45
	32	54
	48	58
	50	
April '94	15	5
	23	10
	24	28
	27	34
	31	45
	32	54
	43	58
	48	
June '94	23	5
	24	10
	27	28
	31	34
	32	45
	48	54
		58
August '94	23	5
	24	10
	27	28
	31	34
	43	45
	48	54
		58
November '94	23	5
	24	10
	27	34
	31	45
	43	54
	48	58
		88
		95
		97

Table 10.3: Variables measured for each female *T. caninus* in control and treatment groups in an experiment at Cambarville, Victoria, to examine the effects of treatment to reduce parasite burdens on growth and survival of young to emergence from the pouch.

Variable
body weight
age-class
haemoglobin (g/dl)
PCV (%)
RCC ($x10^{12}$/l)
WCC ($x10^{9}$/l)
absolute neutrophil count ($x10^{9}$/l)
absolute lymphocyte count ($x10^{9}$/l)
absolute monocyte count ($x10^{9}$/l)
absolute eosinophil count ($x10^{9}$/l)
urea (mmol/l)
bilirubin (mmol/l)
ALP (IU/l)
ALT (IU/l)
GGT (IU/l)
total serum protein (g/l)
albumin (g/l)
globulin (g/l)
glucose (mmol/l)
calcium (mmol/l)
phosphate (mmol/l)

Table 10.4: Presence or absence of dependent young at each capture of female *T. caninus* in an experiment to examine the effects of treatment of adult females with ivermectin and praziquantel to reduce parasite burdens, on the growth and survival of dependent young

Treatment	Animal	Presence of young (+/-) at each capture			
		April	June	Aug	Nov
Treatment	15	+	\	\	\
	23	+	+	+	+
	24	-	+	+	+
	27	+	+	+	+
	31	+	+	+	+
	32	-	+	\	\
	43	-	+	+	-
	48	+	+	+	+
	50	+	+	\	\
Control	5	-	+	+	+
	10	+	+	+	+
	21	-	-	-	-
	28	+	+	+	\
	34	-	+	+	-
	45	-	+	+	+
	54	-	+	+	+
	58	-	+	+	-
	88	\	\	\	+
	95	\	\	\	+
	97	\	\	\	-

\ = animal not caught

Table 10.5: Proportion of adult female *T. caninus* with dependent young in treatment and control groups at each trapping period at Cambarville in an experiment to examine the effects of treatment of adult females with ivermectin and praziquantel to reduce parasite burdens, on the growth and survival of dependent young.

	Proportion of females with dependent young			
	Month			
	April	June	Aug	Nov
Treatment group	0.66 (6/9)	1 (8/8)	1 (6/6)	0.69 (4/6)
Control group	0.25 (2/8)	0.88 (7/8)	0.88 (7/8)	0.60 (6/10)

Figure 10.1: Relationship between head length and age of *T. caninus* pouch young; lines represent the growth curves for each individual.

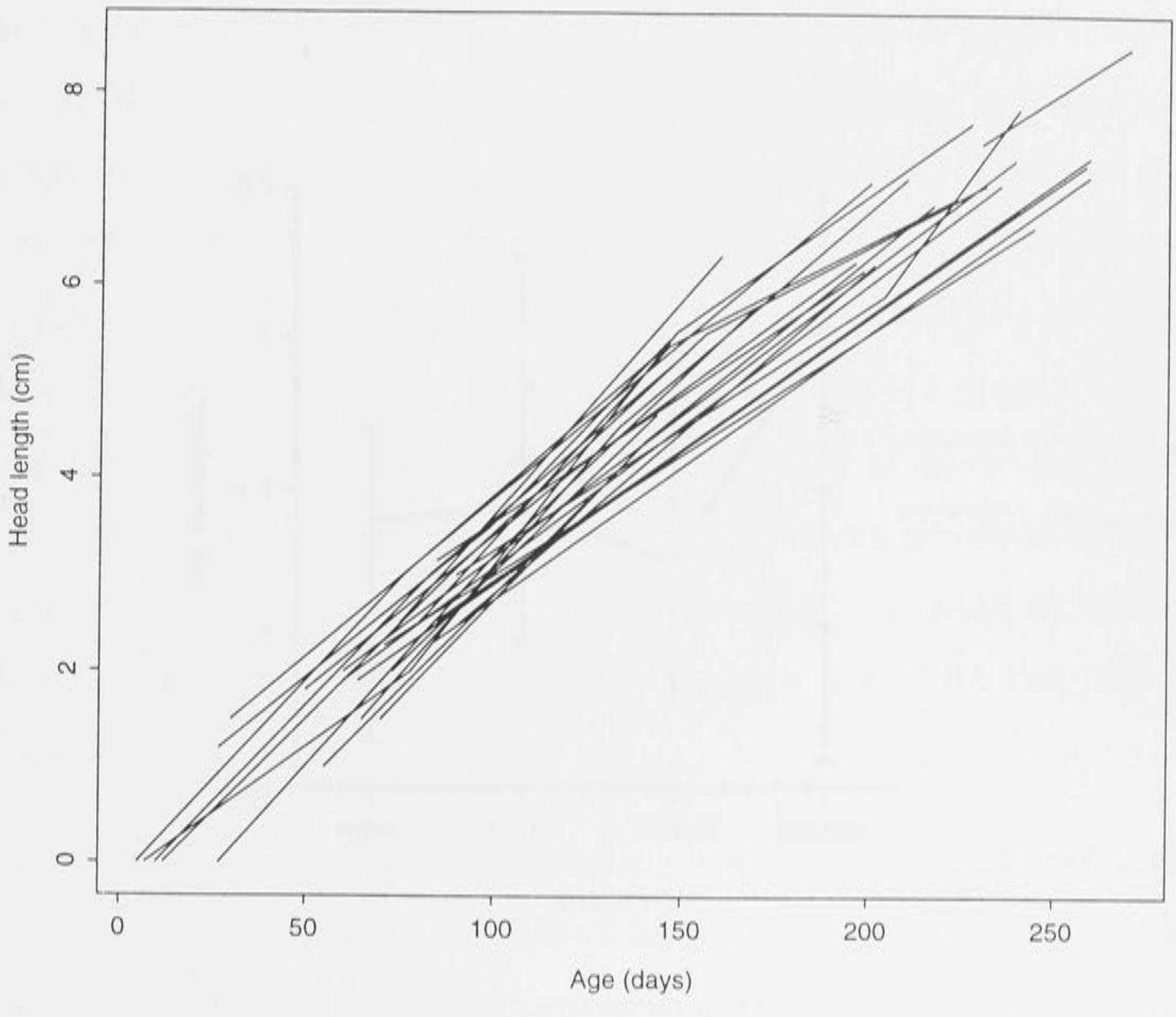
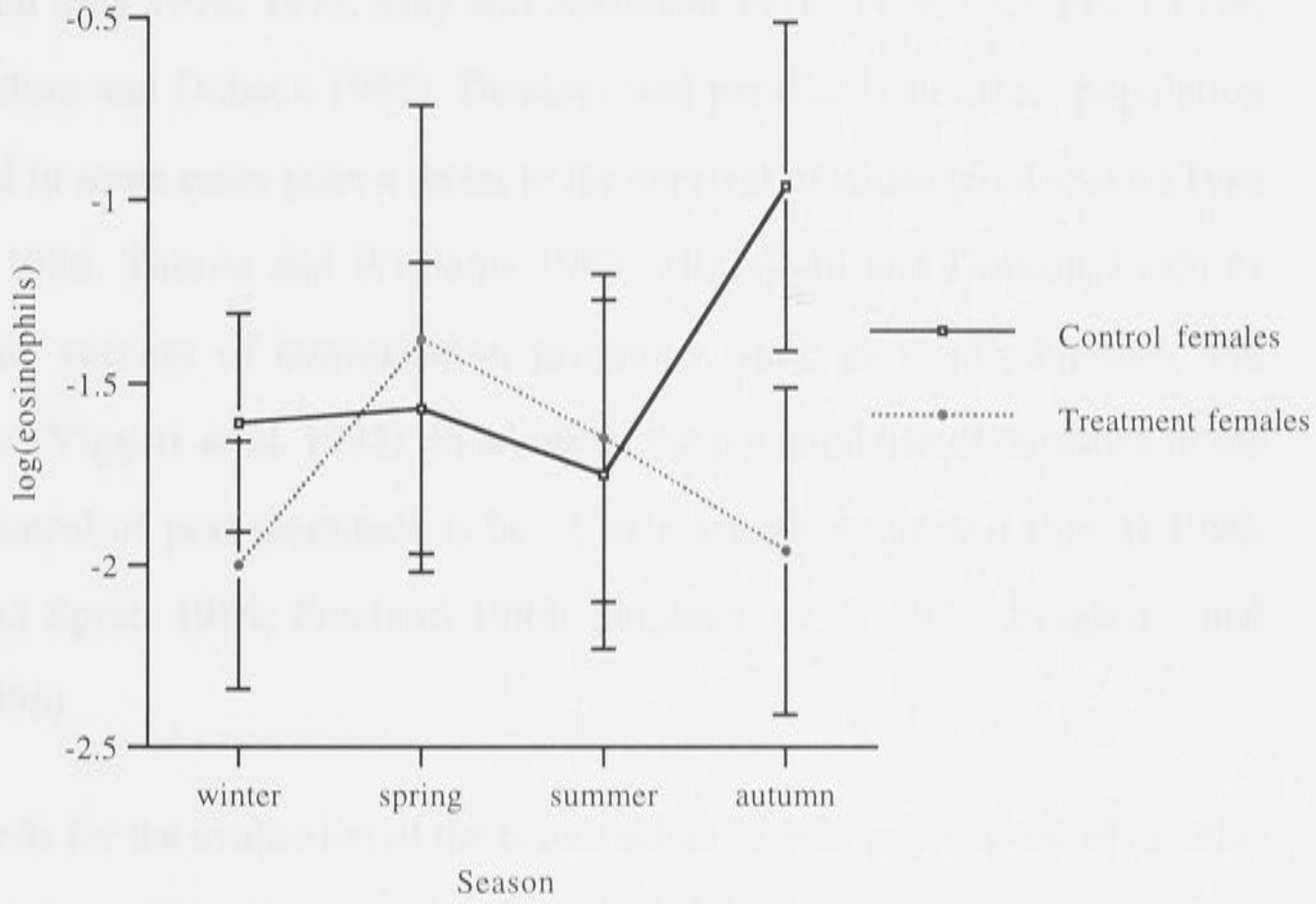


Fig 10.2: Effect on eosinophil counts of treatment with ivermectin to remove parasites from adult female *T. caninus* at Cambarville, Victoria (means and approximate 95% confidence intervals are shown)

p = 0.02



CHAPTER 11

GENERAL DISCUSSION

Parasites may have a major negative impact on their hosts by decreasing survival or reproduction rates, particularly under conditions of nutritional, social or hormonal stress. The importance of the effects and possible regulatory role of diseases and parasites on wild populations of animals has been recognised by many authors (Anderson and May 1978, 1979, May and Anderson 1978, 1979; McCallum 1994, 1995; McCallum and Dobson 1995). Diseases and parasites can affect population dynamics and in some cases pose a threat to the survival of endangered species (van Riper *et al.* 1986; Thorne and Williams 1988; McCallum and Dobson 1995) or jeopardise the success of conservation programs, such as reintroductions and translocations (Viggers *et al.* 1993). In addition, the potential use of parasites in the biological control of pest mammals is being increasingly examined (Spratt 1990; Singleton and Spratt 1986; Freeland 1993; Singleton *et al.* 1995; Singleton and Chambers 1996).

Methods for the evaluation of the health status of wild populations of animals are required to detect ill health and disease in individuals (Whittington and Grant 1983; Canfield *et al.* 1989a). These methods may assist in determining the possible effects of diseases or parasites on health and reproductive capacity of animals. This may be important for a number of reasons including:-

(1) to better understand the epidemiology and transmission of diseases in wild populations;

(2) to record the presence, prevalence and/or effect of a disease agent within a wild population, and assess its importance for populations dynamics and survival (eg. toxoplasmosis in eastern barred bandicoots, *Perameles gunnii,* in Tasmania; Obendorf *et al.* 1994);

(3) to monitor the health and survival of animals which have been translocated or reintroduced to augment declining populations, or into new areas (Holmes 1982; Jones 1982; Haebler 1992; Viggers *et al.* 1993; Woodford 1993; Cunningham 1996);

(4) to examine or monitor the effect of a disease epidemic on natural populations (Langenberg 1992; Munson and Cook 1993), (eg. canine distemper in wild populations of black-footed ferrets, *Mustela nigripes*; Thorne and Williams 1988) ;

(5) to detect exotic disease outbreaks and develop contingency plans to contain and prevent the spread of disease (Geering *et al.* 1995; Hess 1996);

(6) to help identify agents of disease which may be of use in the control of pest species (Spratt 1990);

(7) to inform and guide controlled, tactical releases or introductions of agents of disease in biological control programs (Spratt 1990; Freeland 1993); and,

(8) to provide information on the disease status of wild populations of animals and their potential to function as sources or reservoirs for zoonotic diseases (Mackerras 1954; Smyth 1995);

The first part of this thesis focused on reviewing and applying methods for assessing health and condition of *T. caninus*. The second part of the thesis involved using these methods and approaches to assess and quantify the effects of parasites on *T. caninus* in terms of clinical and subclinical health, and reproductive success. This chapter is a general synthesis of the outcomes of the various aspects of this research.

11.1 SELECTION OF STUDY SPECIES

T. caninus, was selected for this study for several reasons. A number of previous population studies (Owen 1964; How 1972, 1976; Lindenmayer *et al.* 1991a, 1996a, 1996b) provided important ecological and biological information on which to base field studies. In particular, considerable information was available on the population dynamics, trappability, diet and life history of *T. caninus* at Cambarville, in central Victoria (see Chapter 2; Seebeck *et al.* 1984; Lindenmayer *et al.* 1991a, 1996a, 1996b, Warneke *et al.*, unpublished data). This site was selected as the major study location for repeat captures of individuals over different seasons and

for field experiments. The only other site at which *T. caninus* has been studied in detail, [Clouds Creek in northeastern NSW (How 1972, 1976, 1978, 1981; Barnett *et al.* 1979a, 1979b; Presidente *et al.* 1982)], was cleared and replanted with radiata pine, *Pinus radiata*, in the 1970's (How 1972; Barnett *et al.* 1976), and could not be used for further study of the species.

Another reason for selecting *T. caninus* for study was that the species is robust and is easy to capture in wire cage traps. Despite previous extensive trapping of *T. caninus* in other studies, no trap deaths have been recorded (How 1972; Viggers and Lindenmayer 1995; Lindenmayer *et al.*, unpublished data). This was important, given the requirement to trap animals repeatedly and follow animals over prolonged periods of time (see Chapters 4, 6, 9 and 10).

The distribution of *T. caninus* spans a range of habitat types, different climate domains and several biogeographic regions in eastern Australia. This allowed selection of a range of study sites covering regions which would support different intermediate hosts for parasites, thereby providing a basis for detecting parasites with different life-cycle requirements and parasite infracommunity structure. In addition, the patchy distribution of *T. caninus* enabled comparison between geographically discrete populations.

Finally, a detailed investigation of the diversity, abundance and pathogenicity of the parasites of *T. caninus* was important to provide information relevant to the biocontrol of the closely related common brushtail possum, *T. vulpecula* in New Zealand. *T. vulpecula* was introduced to New Zealand in 1851 and is now a pest of ecological and economic importance (Cowan 1991). It has caused extensive damage to indigenous forests (Rose *et al.* 1993), and is both reservoir and vector for *Mycobacterium bovis* (Davidson 1976; Coleman 1988; Jolly 1993). Current methods for control of *T. vulpecula* in New Zealand rely almost entirely on chemical control (poisoning) and shooting. The New Zealand government is directing future research to biological control methods, in the short term through increased biological load of pathogens to reduce reproductive rate, and in the long term by genetic manipulation of

vectors (parasites or viruses) to express DNA coding for factors that could reduce fecundity in the host (Jolly 1993). For example, a genetically modified virus or parasite could produce immunocontraception in *T. vulpecula* by stimulating the host immune system to produce antibodies against some vital physiological process (ie. prevent fertilisation of ova) (Jolly 1993).

The parasites of *T. vulpecula* have been studied more intensively than those of any other Australian marsupial (see Viggers and Spratt 1995, Appendix 1). However, these parasites appear to have few pathogenic effects on the host animal (Viggers and Spratt 1995) and hence may be of limited use for the biocontrol of *T. vulpecula*. Parasites which occur in closely-related species may be more pathogenic when transmitted to *T. vulpecula*. Therefore an improved knowledge of the parasites infecting the closely related *T. caninus* was important as it may provide information and new directions for future research on agents for the biological control of *T. vulpecula*. There are relatively few studies of the parasites of *T. caninus* and there have been no systematic studies of the parasites of this species across its geographical range. Given this, *T. caninus* was considered to be an important host for a detailed and systematic parasitological study at a range of locations throughout its distribution. This aspect of the study aimed to provide information on:- (**1**) the pathogenic effects on animals of parasitic infections, (**2**) the impact of parasites on individual and population health, and, (**3**) which parasites may be potentially useful for biocontrol programs for *T. vulpecula* in New Zealand. These issues are discussed in Part II below.

11.2 PART I

11.2.1 Haematological and serum biochemical analyses

Assessment of the variation in blood values from a standardised reference range is a method that is widely used for the diagnosis of disease in domestic animals (Bush 1991; Duncan *et al.* 1994). Reference haematological and serum biochemical values are also required for wild populations in order to detect or assess the effects of

diseases and/or parasites. Such reference values are available for few species of Australian native marsupials (eg. brown antechinus, *Antechinus stuartii*, Cheal *et al.* 1976; platypus, *O. anatinus*, Whittington and Grant 1983, 1984; eastern quoll, *D. viverrinus*, Melrose *et al.* 1987; koala, *P. cinereus*, Canfield *et al.* 1989a, 1989b; red-tailed phascogale, *P. calura*, Bradley 1990). Blood reference values that are derived from captive populations are unlikely to provide a useful comparison with free-ranging populations of the same species, due to the changed environment, diet, weather conditions and social structure, as well as other stresses of captivity (Middleton 1976).

The interpretation of blood results collected from wild animals must be approached cautiously, because of the wide range of factors which may contribute to variation in these values (see Chapter 4). These may include:- capture stress (Dawson and Denny 1968; Bush 1991), sedation, acclimatisation to repeat captures (Chapple *et al.* 1991), diet (Seal *et al.* 1975; McCue and O'Farrell 1992), social hierarchy, and subclinical disease or parasites (see Chapter 4). Although the potential influence of these factors on blood results may be difficult to quantify, it is important to have knowledge of the variability which may occur in blood values of normal healthy animals within a wild population to enable biologically sensible interpretation of results.

The only previous research on the haematological and serum biochemical values of *T. caninus* was completed by Barnett *et al.* (1979a, 1979b) at Clouds Creek in northeastern NSW. These authors examined the effects of age, sex, season and habitat on a limited number of blood variables (see Chapter 2). In this thesis, a more extensive profile of haematological and serum biochemical variables was investigated in *T. caninus* at seven different study sites (Chapter 4). Reference blood values were established for *T. caninus* at Cambarville in central Victoria (Chapter 4; Appendix 3).

An examination of the variation in blood values of *T. caninus* at several sites across its geographic range, allowed a comparison between populations in different habitats, as well as comparisons within populations. This was important as significant differences in blood values between sites may occur, and comparison with a set of

reference values derived from animals at only one site may not be valid (Canfield *et al.* 1989a).

Knowledge of the ranges of these variables in *T. caninus* was required for interpretation of blood results in relation to other measures of health and estimates of body condition, and to examine effects of parasite prevalence and intensity on blood values. For example, *T. caninus* at Whian Whian State Forest had the highest white cell counts (see Chapter 4) and the prevalence and intensity of infestation with ticks also was higher in animals at this site (see Chapter 7). This suggests that there may be some correlation between these variables. In support of this, the prevalence and intensity of infestation with the ixodid tick *I. holocyclus* was correlated with higher white cell counts (see Chapter 7). The lack of detectable effects of parasites on most other blood values may indicate that:- (**1**) the parasites infecting *T. caninus* were of low pathogenicity or were present at intensities too low to affect host health; or, (**2**) the blood variables measured were not sufficiently sensitive to detect subtle changes in health.

Examination of changes in blood variables in conjunction with other methods of assessment of health and disease, such as histopathological examination (see Chapter 8) enabled a more comprehensive assessment of the pathogenicity of parasites than would otherwise have been possible. Surprisingly, the prevalence and intensity of third stage larvae of the ascaridoid nematode *O. robertsi*, which occurs in the liver of *T. caninus*, was not correlated with elevations of any liver enzymes or metabolites in the peripheral blood (see Chapters 7 and 8).

Despite the large number of factors which may contribute to variation in blood values, haematological and serum biochemical analyses may still be useful to detect ill health and disease (Canfield *et al.* 1989b). Unfortunately, debilitated animals are rarely seen in wild populations because they are susceptible to predation (McCallum 1994; McCallum and Dobson 1995). Consequently, blood tests must be sufficiently sensitive to detect disease in animals that are not in the advanced stages of ill health. Many of the blood variables commonly employed in studies of other animals exhibited

considerable variability in *T. caninus* (see Chapter 4). Canfield *et al.* (1989b) indicated that a wide range of variability may limit the usefulness of haematological and serum biochemical analyses for detecting subtle changes in health of animals. Given this, and on the basis of findings for *T. caninus* in this study, it was considered that a method for quantifying or assessing body condition may be useful to enhance the interpretation of blood values; ie to use in conjunction with blood values to enable a better evaluation of animal health.

11.2.2 Assessment of body condition

A summary and critical examination of methods commonly used to quantify body condition (see Chapter 5) revealed a number of conceptual problems, particularly in the definition of terms. Use of the term "condition" relies on the assumption that those animals considered to be in good "condition" are better able to survive, compete and reproduce in their natural environment than animals in poorer "condition" (Humphreys *et al.* 1984; Krebs and Singleton 1993). "Condition" in this sense, is not easy to measure or quantify, and there may be a wide range of factors contributing to variation in condition, such as:- diet, age, sex, social status, reproductive status, foraging ability, immunological status etc. (see Chapters 5 and 6). This has led to various researchers using methods to estimate body fat, or size to weight ratios (condition indices) to quantify condition (see Chapter 5; Bamford 1970; Presidente *et al.* 1982; Garrow 1983; Kie *et al.* 1983; McCown *et al.* 1991; Krebs and Singleton 1993). These methods are based on the key assumption that animals carrying higher levels of body fat are in better condition, both physiologically and ecologically, than those with smaller fat reserves (Krebs and Singleton 1993; Humphreys *et al.* 1984). This assumption also may be flawed (see below).

Traditionally, condition "indices" based on measures of body mass and skeletal size have been widely accepted as a method of scoring condition in wild animals (Bailey 1968; Bakker and Main 1980; Angerbjörn 1986; Krebs and Singleton 1993). Geometric scaling theory suggests that body mass should be related to the cube of length (Krebs and Singleton 1993), and many authors have derived a condition index

by dividing body mass by a measure of length cubed (Bailey 1960; Angerbjörn 1986; Lehmann 1992; Short and Turner 1994; Chastel *et al.* 1995). However, this approach may not be valid for all species. A condition index was derived for *T. caninus* from a regression of the relationship between body mass and body length measurements from 104 animals from seven sites across the geographic range of the species. This relationship for *T. caninus* was body mass / body length^2, which differed from geometric scaling theory and findings for other species. This shows that, if condition indices are to be used, a direct examination of the relationship between body mass and a measure of skeletal size should be completed for each species.

To test the assumption that condition index reflects fat deposits in animals, this index for *T. caninus* was validated using dilution of isotopic water as an indirect estimate of body fat. There was no significant relationship between condition index and body water composition, which indicated that the condition index did not reflect body fat depots in *T. caninus* at Cambarville. In addition, there was some field evidence from long term breeding and population data that "condition", defined in terms of fat depots, did not necessarily reflect "fitness". For example, "fatter" animals may not always breed. Adult female C21 at Cambarville was captured over four years, but never reproduced successfully. The body water composition of this animal was low compared to other animals at Cambarville (ie. C21 was "fatter" than most other animals). Females that are thinner may have lower fat depots because they contribute a higher level of maternal investment to the rearing of their young and thus may be more likely to successfully reproduce than fatter females. This emphasises the difficulty in defining, quantifying and inter-relating "fitness" and "condition".

In conclusion, considerably more work and discussion is required to determine a more precise definition of the term "condition" for wildlife *per se*. More specific methods are required to quantify or assess both health and condition. It is likely that a combination of factors will need to be determined to develop an overall assessment of fitness or condition. This may include a measure of reproductive success, such as the

number of young produced and reared over a specified period for female animals or number of young fathered by males.

11.3 PART II

Having established and evaluated some of the readily available and widely used methods for determining health and condition of animals, the second part of the thesis aimed to use these tools, where applicable, to assist in investigating the effects of parasites on their hosts, using *T. caninus* as a case study. This aspect of the study was completed in two sections. First, the parasite fauna of *T. caninus* was examined at several study sites across the host's geographic range (Chapters 7 and 8). This provided information on the structure of communities of parasites occurring in *T. caninus* across its range and on the pathogenic effects associated with parasitic infection. Secondly, based on these results, a field-based parasite burden manipulation experiment was completed to investigate the effects of helminths and ectoparasites on host population regulation (Chapter 10).

11.3.1 Pathological changes associated with parasitic infection

The effects of individual parasite species on host tissues can be examined by gross and histopathological examination of organs at necropsy. This can provide some information on the virulence and pathogenicity of parasites and an understanding of the pathological and physiological mechanisms by which a parasite species may affect host health. Of the parasites that were recorded from *T. caninus*, *M. minesi* and *O. robertsi* appeared to have the greatest potential to exert a harmful effect on the host.

Heavy infestation of the lungs with *M. minesi* caused damage to respiratory parenchyma, and may reduce host fitness by decreasing respiratory capacity, possibly increasing susceptibility to predation (see Holmes 1995). This parasite was of intermediate pathogenicity and its effects on host tissues appeared to be density dependent (ie. pathological changes were more pronounced when the parasite was present at higher intensities, see Chapter 8). McCallum (1994) suggested that such parasites were most likely to have a regulatory effect on host populations. Patterns of

dispersion of parasites within hosts also may influence the ability of parasites to regulate host populations (Anderson and May 1978). Parasites present at high prevalences are unlikely to have a major effect on the host population (Anderson and May 1978; McCallum 1994). *M. minesi* was present at an intermediate prevalence (50%) in populations of *T. caninus*. Despite the potential of this parasite for regulation of host populations, it could not be included in the field manipulation experiment due to difficulties in detecting this parasite in the living host. The use of the Baermann technique for collecting lungworm larvae was discontinued due to failure to detect larvae, even in populations where adult *M. minesi* were found in necropsied animals (see Chapter 3.8.3). Treatment of animals with ivermectin would have removed metastrongyloid nematodes if present, however it was not possible to determine presence or intensity of infection prior to treatment. Despite this, *M. minesi* warrants further investigation as a parasite species which may have an effect on host population regulation.

The presence of third stage larvae of *O. robertsi* in the liver of *T. caninus* was also associated with significant pathological changes in host tissues. The long term effects of this parasite on the health and survival of *T. caninus* are unknown. In those host populations in which it occurred, *O. robertsi* was present at a prevalence of approximately 25%; and it is possible that it may have some impact on the population structure and dynamics of *T. caninus*. Further studies are required to determine whether the presence of *O. robertsi* is associated with any changes in the age structure of infected populations of *T. caninus*. McCallum (1994) suggested that if parasites were most prevalent in hosts of intermediate ages, and if the degree of parasite aggregation in hosts was found to decrease with age, this would provide evidence of mortality due to that parasite species.

The filarioid nematode *S. venacavincola* was also present at intermediate prevalence (22-57%) in those host populations in which it was detected (see Chapter 7). Diagnosis of infection with this parasite is possible in living animals, however quantification of the level of infection is difficult. The fecundity of parasites may be

modified by the host immune response (Anderson 1979, Freeland 1986), so that the number of microfilariae circulating in the blood may not reflect the intensity of infection with adult female *S. venacavincola*. Knowledge of the intensity of infection would be advantageous, as the effects of this parasite on the host are probably density dependent. *S. venacavincola* is found in the caudal vena cava and hepatic veins (Spratt and Varughese 1975), and when present in large numbers could cause significant disruption to blood flow in these major vessels, as occurs in heartworm (caused by *Dirofilaria immitis*) in the dog, *Canis familiaris*, (Sutton 1988) and elaeophorosis (caused by *Elaeophora schneideri*) in elk, *Cervus canadensis* (Adcock *et al.* 1965; Adcock and Hibler 1969). Given this, the possible effect of *S. venacavincola* on the health of *T. caninus* warrants further investigation.

Diffuse peritonitis has been recorded from one *T. caninus* from which 45 *B. trichosuri* were collected, indicating that heavy burdens of this parasite may be pathogenic (Beveridge in Presidente 1984). However, in this study, there was only one record of a single specimen of *B. trichosuri* from *T. caninus* in central Queensland. Although it appears that this parasite may have a significant pathogenic effect on its host, parasites that are present at very low prevalences (ie. highly aggregated) are unlikely to have a regulatory effect on host populations (Anderson and May 1978; McCallum 1994). Therefore, this parasite species may be of less interest for further studies, despite its potentially high levels of pathogenicity.

11.3.2 Communities of parasites of *T. caninus*

In natural populations, hosts are generally subject to multispecies parasitic infections that may lead to additive or synergistic depression of host fitness (Freeland 1986; Holmes 1995). Examination of pathological changes associated with the presence of individual parasite species provides information about the effects of parasite on host tissues, but gives only limited insight into the effects of parasites on host death rate or fecundity (McCallum and Dobson 1995; Grenfell and Gulland 1996). A consideration of the potential effect on the host of the total parasite infracommunity is important, given that most parasites are subject to density

dependent constraints and inter- and intraspecific competition (Anderson and May 1978; Freeland 1986). Field-based experiments in which host parasite populations are manipulated, can be used to test the effects of parasites on host population dynamics (Chapter 10). This allows an examination of the effect of these parasites on individual hosts in terms of health and reproductive success. This type of information is important to ascertain whether specific parasites or parasite communities are able to exert a regulatory effect on host populations, thereby enabling an assessment of:- (**1**) the importance of parasites in conservation programs, (eg. in the case of an endangered species); or, (**2**) the potential usefulness of parasites for the biocontrol of pest mammals.

The species richness of parasite component communities in *T. caninus* was low in comparison with a number of other marsupials and mammals (see Chapter 7). This is probably a consequence of the diet, foraging behaviour and habitat of this host. The dietary items consumed by *T. caninus* probably do not include many organisms that could function as intermediate hosts for parasites. Despite the foraging habits of *T. caninus*, which includes some time feeding on the ground, this host is probably not exposed to infective stages of many parasites. Alternatively, *T. caninus* may have been exposed to additional parasite species which have failed to colonise this host.

There is limited knowledge of the life-cycles of the helminth parasites infecting *T. caninus* (see Appendix 1, Viggers and Spratt 1995). Species that commonly co-occur may have similar life-cycle requirements, or may share the same, or a similar, intermediate host (Bush and Holmes 1986). No clear patterns of co-occurrence among helminth species were recorded for *T. caninus* from seven study sites (see Chapter 7). Further studies on individual parasite species are required to gain an improved understanding of parasite transmission and life-cycles in this host. In particular, this type of knowledge could be useful to attempt field-based experimental augmentations of infections with potentially pathogenic helminth species, to examine their effects on host health and fecundity.

11.3.3 Implications for biocontrol of *T. vulpecula* in New Zealand

The common species of gastrointestinal nematodes which infect *T. caninus* across most of its distribution are also present in *T. vulpecula* in New Zealand. Few pathological changes have been recorded in association with the presence of these parasite species in either host (Chapter 7 and 8; Viggers and Spratt 1995, Appendix 2). Parasites occurring in *T. vulpecula* and *T. caninus* in Australia which require intermediate hosts to complete their life-cycles are absent in New Zealand, with the notable exception of *Bertiella trichosuri*. The metastrongyloid nematode, *M. minesi*, and the filarioid nematodes *S. venacavincola* and *Brienlia trichosuri* are not present in *T. vulpecula* in New Zealand. The intermediate hosts for these parasites are currently unknown, but are probably also absent from New Zealand. These parasite species were recorded from *T. caninus* captured in subtropical rainforest gullies and habitat patches. Thus it appears unlikely that the intermediate hosts for these parasites would survive in the more temperate southern climate of New Zealand. Further studies of the life cycle and pathogenicity of these parasites are required to assess their effects on host health and fitness and its potential usefulness as an agent of biocontrol.

Although *O. robertsi* caused considerable pathological changes in the liver of infected *T. caninus*, this parasite is unlikely to be useful for control of *T. vulpecula* in New Zealand. This is because the definitive host for this parasite (the carpet python, *Morelia spilotes variegata*) does not occur in New Zealand.

11.3.4 Parasite manipulation experiment

Hypotheses on the regulation of populations by parasites can be tested in the field by comparing the survival and fecundity of "control" animals, with animals in which disease and parasite burdens have been manipulated (Krebs 1995; McCallum 1994, 1995; McCallum and Dobson 1995). Prior to completing this type of study for *T. caninus*, a drug trial was conducted to determine the efficacy of ivermectin and praziquantel for removing or reducing parasite burdens in this host species. Although the efficacy of praziquantel could not be easily assessed, treatment with ivermectin

removed parasites from *T. caninus* within ten days. Animals were re-infected with parasites and shedding eggs in the faeces within eight weeks of treatment with ivermectin. This indicates that either the pre-patent periods of gastrointestinal helminths in *T. caninus* were less than eight weeks, or that ivermectin did not kill dormant or immature nematodes, which had matured during the period since treatment and started to shed eggs.

Although the species richness of helminth species was lower at Cambarville than some other study sites, the Cambarville population was selected for study given the historical knowledge of individuals from previous studies, the high population density and known trappability of animals at this site. A field-based parasite manipulation experiment to examine the effects of parasites on reproductive success of *T. caninus*, showed that the parasites infecting *T. caninus* at Cambarville did not appear to affect birth rate, death rate or growth rate of pouch-dependent young. However, sample sizes were small, which reduced the power of the experiment for detecting small changes in reproductive success. It is possible that the low number of parasite species occurring in *T. caninus* at Cambarville may have influenced the likelihood of detecting a response to treatment. However, given that some of the additional helminth species present at other study sites were detected at low prevalences and intensities (eg. *Brienlia trichosuri* and *Gongylonema* sp.), their contribution to variation in population dynamics also may have been difficult to detect.

Reduction of parasite burdens did not have any significant effect on most haematological and serum biochemical variables of pregnant or lactating *T. caninus*. Alternatively, the blood tests used may not have been sufficiently sensitive to detect the effects of parasites. The findings of this study suggest that the parasite species infecting *T. caninus* at Cambarville were probably of low pathogenicity and unlikely to exert a regulatory effect on the host population.

Numerous characteristics of wild populations may make it difficult to detect the effects of parasites. Such factors may include:- habitat variability, population density and heterogeneity in host diet, social status, genetics, behaviour and susceptibility to

disease (Holmes 1982, 1995). Ideally, an experiment to detect the effects of parasites on their hosts in wild populations would require a large number of host individuals of similar age, size etc., living in an area of homogeneous habitat quality. This reduction in heterogeneity would eliminate some of the problems experienced in separating the effects of parasites from the multitude of other factors which may affect host survival, birth and death rates and host health. For this reason, most studies on the effects of parasites have been completed on captive populations of animals. Despite the complexity of processes acting on wild populations, continued research is required to attempt to describe the effects of parasites on their wild hosts, given that the captive situation is markedly different from the natural environment.

11.4 CONCLUSIONS

(1) Reference haematological and serum biochemical values were established for *T. caninus* at Cambarville in Victoria.

(2) Significant differences between sexes, seasons and site location were detected for several haematological and serum biochemical values of *T. caninus*. This variation should be taken into account when collecting and interpreting blood values of *T. caninus* in field studies.

(3) Considerable variability was evident in the haematological and serum biochemical values of *T. caninus*, indicating that subtle changes in health are unlikely to be detected. Other methods are required for assessing health in free-ranging populations of *T. caninus* that are more sensitive to ill-health, or that can be used in conjunction with blood values to gain a better assessment of health or condition.

(4) Condition indices were derived for *T. caninus* from a regression of the relationship between body mass (kg) and body length (cm). This relationship for *T. caninus* was body mass / length^2. This differed from the findings of numerous studies of other species in which this relationship was body mass / length^3. If condition indices are to be used to quantify body condition, they should be independently derived for each species.

(5) Dilution of isotopic water in *T. caninus* to give an indirect measure of body fat showed that condition indices for this species were not correlated with body fat. The assumption that condition indices reflect body fat is invalid for *T. caninus* at Cambarville. More consideration must be given to definitions of condition and fitness, and methods for assessing health and condition in free-ranging populations.

(6) Considerable variation was found among the helminth and arthropod ectoparasite component communities of *T. caninus* at seven study sites across the host's geographic range. Differences were detected for several parasite species in prevalence, intensity of infection and species richness. No significant associations between parasite species were found. There was evidence for co-evolution of some parasite species with this host, and probable host-switching for other parasite species.

(7) Pathological changes were found in association with several helminth species in *T. caninus*; particularly *O. robertsi* and *M. minesi*. Unidentified foreign bodies were associated with the formation of multiple fibrogranulomas and extensive damage to respiratory epithelium in the lungs of two *T. caninus* at Cambarville. This damage may be caused by a parasite and warrants further investigation.

(8) There was little serological evidence of exposure of *T. caninus* to various agents of disease. *T. caninus* from seven populations across the geographic range of the species had negative titres for *Leptospira interrogans* serovar *hardjo*, which cross-reacts with antibodies to *L. interrogans* serovar *balcanica*. *Balcanica* infection is endemic in populations of *T. vulpecula*, so its absence in *T. caninus* was surprising.

(9) Ivermectin successfully reduced helminth infections in three *T. caninus* at Cambarville. Animals were re-colonised by parasites and shedding eggs in the faeces within eight weeks of treatment. Re-infestation with ectoparasites of two *T. caninus* treated with ivermectin did not occur within 10 days of treatment.

(10) Treatment of adult female *T. caninus* to reduce helminth and ectoparasite burdens did not significantly affect the birth rate, survival or growth rates of dependent young.

(11) The parasites which occur in *T. caninus* at Cambarville appear to be of low pathogenicity and are unlikely to play a role in the regulation of this host population.

11.5 FURTHER RESEARCH

There is a paucity of research on wild populations of mammals to examine the effects of parasites on their hosts (see Grenfell and Gulland 1996). Further studies need to be conducted in the form of field experiments to further explore the concept of host population regulation by parasites (Freeland 1990; Krebs 1995; McCallum 1994, 1995). An expanded study of *T. caninus* at Cambarville over a longer time period may provide clearer information on the effects of parasites on the species. Alternatively, a study of a population of *T. caninus* with a greater diversity of parasites may be more likely to detect significant effects of parasites on host population regulation or host health.

Field-based experiments to augment parasite burdens, rather than reduce them, may provide greater insight into the effects of parasites on hosts. Further studies on the transmission and life-cycles of potentially pathogenic helminth species which infect *T. caninus*, such as *M. minesi* and *S. venacavincola,* are required to enable delivery of infective stages of parasites to experimental hosts. It is possible that the long life expectancy of *T. caninus* may contribute to difficulties in detecting effects of parasites over short time frames. Therefore, it may be advantageous to conduct field experiments on a mammal species which has a shorter life span and a greater diversity of parasites (eg. *Antechinus* spp.).

Given that the effects of parasites which are endemic in populations may be subtle and difficult to detect, better methods are required to evaluate health and condition in hosts. Tests to quantify immune competence and resistance to disease also may be considered as measures of fitness. Prospective methods must be tested on both captive and wild populations to evaluate their sensitivity and usefulness.

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APPENDIX 1

Wildl. Res., 1995, **22**, 311–32

The Parasites Recorded from *Trichosurus* Species (Marsupialia : Phalangeridae)

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Abstract

This review outlines the known endoparasites and ectoparasites of *Trichosurus vulpecula* and *T. caninus* in Australia and *T. vulpecula* in New Zealand. Any associated gross and histopathological changes in the host that occur because of parasite infection are also described. Endoparasites from the Protozoa, Cestoda and Nematoda are found in both *T. vulpecula* and *T. caninus*. However, the trematode, *Fasciola hepatica*, has been recorded only from *T. vulpecula*. Infection of *T. vulpecula* with this parasite has been associated with extensive pathological changes in the liver. Numerous species of fleas, ticks and mites occur on *T. caninus* and *T. vulpecula* in Australia, but only mites occur in New Zealand; ticks and fleas are absent. The only parasite with an indirect life cycle that occurs in *T. vulpecula* in New Zealand is the cestode *Bertiella trichosuri*. Other parasites that require intermediate hosts to complete their life cycle, such as the metastrongyloid and filarioid nematodes, are absent. Further studies are required to investigate the effects of parasites on the health and fecundity of *T. vulpecula* and *T. caninus* in Australia to determine their potential as direct or indirect agents for biological control of *T. vulpecula* in New Zealand.

Introduction

The parasites of the common brushtail possum, *Trichosurus vulpecula*, have been recorded over many parts of the range of the species both in Australia, where it is native (Mackerras 1959; Doube 1975; Beveridge 1976, 1978; Presidente 1984; O'Callaghan and Moore 1986), and in New Zealand (Bowie and Bennett 1983; Warburton 1983), where it was introduced in 1851 and has since attained pest status (Cowan 1990). The mountain brushtail possum, *T. caninus*, has been studied at relatively few locations (How 1972, 1976, 1978, 1981; Barnett *et al.* 1979*a*, 1979*b*) and detailed studies of the parasites of *T. caninus* have been undertaken at a single study site at Clouds Creek in north-eastern New South Wales (Presidente *et al.* 1982; Presidente 1984).

This review outlines the known endoparasites and ectoparasites of the *Trichosurus* species in Australia and New Zealand, and the pathological changes associated with them. This provides current baseline information from which to direct research on parasites in relation to biocontrol of *T. vulpecula* in New Zealand. Taxonomic authorities for parasite species and distribution records are given in Tables 1–4.

Abbreviations

Abbreviations used in this paper are as follows: ACT, the Australian Capital Territory; NSW, New South Wales: NT, the Northern Territory; NZ, New Zealand; Qld, Queensland; SA, South Australia; Tas., Tasmania; Vic., Victoria; WA, Western Australia.

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Table 1. Endoparasites of *Trichosurus vulpecula* and locations in which they were recorded

Parasite species	Australia						NZ
	Vic.	NSW	SA	WA	Tas.	Qld	
Protozoa							
Eimeriidae							
Eimeria spp.	+				+		+
Toxoplasma gondii Nicolle and Manceaux 1909	+					+	
Sarcocystidae							
Sarcocystis spp.	+						
Endamoebidae							
Entamoeba sp.						+	
Cestoda							
Anoplocephalidae							
Bertiella trichosuri Khalil 1970	+	+	+		+		+
Trematoda							
Fasciolidae							
Fasciola hepatica Linnaeus 1758	+	+			+		+
Nematoda							
Rhabditoidea							
Strongyloididae							
Parastrongyloides trichosuri Mackerras 1959		+				+	+
Strongyloides sp.		+					
Trichostrongyloidea							
Herpetostongylidae							
Paraustrostrongylus trichosuri Mawson 1973	+	+	+			+	+
Trichostrongylidae							
Trichostronglyus colubriformis (Giles 1892)		+	+				+
Trichostrongylus axei (Cobbold 1879)			+				
Trichostrongylus rugatus Monnig 1925		+					
Trichostrongylus retortaeformis (Zeder 1800)							+
Trichostrongylus vitrinus Looss 1905		+					
Trichostrongylus sp.	+	+	+				+
Cooperia curticei (Giles 1892)		+					
Molineidae							
Nematodirus sp.		+					
Dromaeostrongylidae							
Filarinema trichosuri (Johnston and Mawson 1939)		+					
Peramelistrongylus sp.		+					
Profilarinema hemsleyi Durette-Desset and Beveridge 1981			+				
Profilarinema sp.		+			+		
Metastrongyloidea							
Angiostrongylidae							
Marsupostrongylus minesi Spratt 1979		+					
Marsupostrongylus pseudominesi Spratt 1984						+^	
Marsupostrongylus longilarvatus Spratt 1979		+					
Filostrongylus tridendriticus Spratt 1984					+		
Oxyuroidea							
Oxyuridae							
Adelonema trichosuri (Johnston and Mawson 1938)	+	+	+			+	
Ascaridoidea							
Ascarididae							
Ophidascaris robertsi (Sprent and Mines 1960)		+					
Spiruroidea							
Gongylonematidae							
Gongylonema sp.		+	+		+		
Spiruridae							
Protospirura marsupialis Baylis 1927		+				+	
Filarioidea							
Onchocercidae							
Breinlia trichosuri (Breinl 1913)		+				+	
Sprattia venacavincola (Spratt and Varughese 1975)		+				+	

^Found in *Trichosurus vulpecula johnstoni*.

Table 2. Endoparasites of *Trichosurus caninus* and locations in which they were recorded

Parasite species	Vic.	NSW	Qld
Protozoa			
Eimeriidae			
Eimeria sp.		+	
Sarcocystidae			
Sarcocystis spp.		+	
Cestoda			
Anoplocephalidae			
Bertiella trichosuri Khalil 1970	+	+	
Nematoda			
Rhabditoidea			
Strongyloididae			
Parastrongyloides trichosuri Mackerras 1959		+	
Trichostrongyloidea			
Herpetostrongylidae			
Paraustrostrongylus trichosuri Mawson 1973	+	+	
Dromaeostrongylidae			
Filarinema trichosuri (Johnston and Mawson 1939)		+	
Metastrongyloidea			
Angiostrongylidae			
Marsupostrongylus minesi Spratt 1979		+	+
Oxyuroidea			
Adelonema trichosuri (Johnston and Mawson 1938)		+	
Ascaridoidea			
Ascarididae			
Ophidascaris robertsi (Sprent and Mines 1960)		+	
Filarioidea			
Onchocercidae			
Breinlia trichosuri (Breinl 1913)		+	
Sprattia venacavincola (Spratt and Varughese 1975)		+	+

Endoparasites

Protozoa

Eimeriidae

Eimeria spp. Oocysts of an undescribed species of *Eimeria* have been reported from the faeces of *T. vulpecula* (Presidente 1982, 1984) and *T. caninus* (Presidente *et al.* 1982). High numbers of oocysts were detected in faecal examinations of juvenile *T. vulpecula* and of adult females with advanced pouch young or early back young (Presidente 1982). However, this eimerian species was considered to be of low pathogenicity, as large numbers of oocysts in the faeces of experimentally infected animals were not accompanied by diarrhoea, and post-mortem examination of the intestinal tract showed only mild pathological changes (Presidente 1982). O'Callaghan and Moore (1986) detected oocysts in faecal samples from eight male and three female subadult and adult *T. vulpecula* on Kangaroo Island, SA. Unidentified coccidian oocysts have also been detected in the faeces of *T. vulpecula* from Wallaceville and Lincoln in NZ (Clark, unpublished data).

Coccidian oocysts were detected in the faeces of *T. caninus* from Clouds Creek, north-eastern NSW (Presidente *et al.* 1982). These oocysts have not been described, and they may be identical to those found in *T. vulpecula* (Presidente 1984).

Toxoplasma gondii. *T. gondii* is a protozoan parasite of the cat, *Felis catus*. Numerous species of mammals, including marsupials, may function as intermediate hosts for

Table 3. Ectoparasites of *Trichosurus vulpecula* and locations in which they were recorded

Parasite species	Australia						NZ
	Vic.	NSW	SA	WA	Tas.	Qld	
Acari (ticks)							
Ixodoidea							
Argasidae							
Ornithodoros macmillani (Hoogstraal and Kohls 1966)						+	
Ixodidae							
Ixodes holocyclus Neumann 1899		+				+	
Ixodes cornuatus Roberts 1960					+		
Ixodes fecialis Warburton and Nuttall 1909							
Ixodes hirsti Hassall 1931		+			+	+	
Ixodes tasmani Neumann 1899	+	+^	+	+	+	+	
Ixodes trichosuri Roberts 1960		+			+		
Haemaphysalis bancrofti Nuttall and Warburton 1915		+				+	
Haemaphysalis bremneri Roberts 1963						+	
Haemaphysalis humerosa Warburton and Nuttall 1909		+				+	
Haemaphysalis ratti Kohls 1948						+	
Acari (mites)							
Mesostigmata							
Laelapidae							
Trichosurolaelaps crassipes Womersley 1956	+	+	+		+	+	+
Haemolaelaps sisyphus Domrow 1981		+				+	
Laelapsella humi Womersley 1955	+						
Sarcoptiformes							
Atopomelidae							
Atellana papilio Domrow 1958		+			+	+	+
Petrogalochirus dycei Domrow 1960	+	+^					+
Murichirus sp.							+
Echimyopodidae							
Marsupiopus trichosuri Fain 1968		+^					
Sarcoptidae							
Notoedres muris (Megnin 1877)	+						
Trombidiformes							
Trombiculidae							
Ascoschoengastia rattus (Womersley and Heaslip 1943)	+			+	+	+	
Eutrombicula hirsti (Sambon 1927)						+	
Neotrombicula novaehollandiae (Hirst 1929)					+		
Guntheria peregrina (Womersley 1952)						+	
Guntheria trichosuri (Womersley 1939)						+	
Guntheria pseudomys (Womersley 1952)						+	
Guntheria shieldsi (Gunther 1941)						+	
Guntheria kallipygos (Gunther 1939)					+		
Trombicula quadriensis Womersley and Heaslip 1943					+	+	
Prostigmata							
Leeuwenhoekiidae							
Odontacarus sp.						+	
Siphonaptera (fleas)							
Pulicidae							
Echidnophaga myrmecobii Rothschild 1909	+	+	+	+			
Pygiopsyllidae							
Acanthopsylla pavida (Rothschild 1916)						+	
Acanthopsylla rothschildi rothschildi ((Rainbow 1905)	+	+			+		
Choristopsylla ochi (Rothscild 1904)	+	+	+	+	+	+	
Choristopsylla tristis (Rothschild 1900)						+	
Bibikovana hoplia (Jordan and Rothschild 1922)	+				+		
Pygiopsylla sp.						+	

^Also recorded in ACT.

Table 4. Ectoparasites of *Trichosurus caninus* and locations in which they were recorded

Parasite species	Vic.	NSW	Qld
Acari (ticks)			
Ixodoidea			
Ixodidae			
Ixodes holocyclus Neumann 1899		+	+
Ixodes hirsti Hassall 1931	+		
Ixodes tasmani Neumann 1899		+	+
Ixodes trichosuri Roberts 1960		+	
Acari (mites)			
Mesostigmata			
Dermanyssidae			
Trichosurolaelaps crassipes Womersley 1956		+	+
Trichosurolaelaps dixous Domrow 1972		+	+
Haemolaelaps penelope Domrow 1964		+	+
Sarcoptiformes			
Atopomelidae			
Atellana papilio Domrow 1958		+	
Petrogalochirus dycei (Domrow 1960)		+	
Trombidiformes			
Trombiculidae			
Ascoschoengastia rattus (Womersley and Heaslip 1943)		+	
Guntheria kallipygos (Gunther 1939)			+
Guntheria lappacea (Womersley 1952)			+
Trombicula quadriensis Womersley and Heaslip 1943			+
Siphonaptera (fleas)			
Pygiopsyllidae			
Acanthopsylla pavida (Rothschild 1916)		+	

T. gondii (Smith and Munday 1965; Charleston 1994). Oocysts are shed in the faeces of infected cats, and animals are usually infected by the ingestion of oocyst-contaminated food. In the intermediate host, *T. gondii* usually forms cysts in the brain and muscle tissue. Clinical signs may develop, such as loss of co-ordination, circling, blindness, convulsions and death.

Although *T. vulpecula* feeds commonly on the ground in some areas (Pullan and Mansfield 1974; Tyndale-Biscoe 1979; Green and Coleman 1980, 1981; O'Callaghan and Moore 1986), *T. gondii* has been recorded in *T. vulpecula* only in Qld (Cook and Pope 1959) and Vic. (Presidente 1982). Serum antibodies to *T. gondii* were not detected in 40 *T. vulpecula* inhabiting areas adjacent to grazing land on Kangaroo Island, SA (O'Callaghan and Moore 1986). Similarly, *T. gondii* has not been detected in other studies of *T. vulpecula* in Tas., Qld or NZ (Smith and Munday 1965; Munday 1969).

Sarcocystidae

Sarcocystis spp. These are protozoan parasites that are sought rarely and thus detected only occasionally in skeletal muscle tissues of marsupials (Munday *et al.* 1978). In these hosts, sarcocysts are typically thin-walled and contain small zoites (Munday *et al.* 1978). Munday *et al.* (1978) reported the presence of numerous cysts in the tongues of two of 44 *T. caninus* from Clouds Creek in north-eastern NSW (see also Presidente *et al.* 1982). There is only one published record of an infection in *T. vulpecula*: sarcocysts were detected in the extrinsic muscles of the eye in one of 155 animals collected from south-eastern Australia (Munday *et al.* 1978). Sarcocysts were not observed in histological sections of the tongues of four *T. vulpecula* from south-eastern NSW, two from Timbillica State Forest (37°26'S, 149°55'E), one from Bondo State Forest (36°21'S, 149°09'E), and one from Nimmo Hill

(36°11'S, 148°38'E) (Spratt, unpublished data). *Sarcocystis* spp. may not have been searched for in populations of *T. vulpecula* in NZ.

Endamoebidae

Entamoeba sp. Endamoebidae are parasites of the digestive tract of invertebrates and vertebrates. Four major groups of the genus *Entamoeba* are recognised (*histolytica, coli, bovis, gingivalis*), with *E. histolytica* the only species considered seriously pathogenic to mammals. Mackerras (1958) reported *Entamoeba* sp. from *T. vulpecula* in Qld, but it is unlikely that other workers have examined gastrointestinal tracts or faeces for this parasite.

Trematoda (Flukes)

Fasciolidae

Fasciola hepatica. *F. hepatica*, the common liver fluke of sheep, *Ovis ovis*, and cattle, *Bos taurus*, is one of the more pathogenic parasites in Australian native mammals (Spratt and Presidente 1981). The occurrence of *F. hepatica* is dependent on the presence of a suitable environment for eggs to hatch and for the hatched miracidia to infect the snail intermediate hosts, predominantly *Austropeplea tomentosa* but also *A. columnella*. The parasite is widespread in temperate, agricultural regions and in swampy areas associated with creeks in south-eastern Australia (Boray 1969; Spratt and Presidente 1981). Cercariae passed by infected snails encyst as metacercariae on fresh green vegetation where they may be ingested by grazing marsupials (Spratt and Presidente 1981).

A low prevalence (5%) of natural infection with *F. hepatica* was reported in *T. vulpecula* in south-eastern NSW (Spratt and Presidente 1981). This may be attributable to (1) the comparatively low population density of *T. vulpecula* in its native environment, and (2) the predominantly arboreal habits of this species in south-eastern mainland Australia, rendering it unlikely to come in contact with the encysted metacercariae (Spratt and Presidente 1981). *F. hepatica* has also been recorded in *T. vulpecula* in NZ, where *T. vulpecula* spends a considerable amount of time feeding on the ground (Tyndale-Biscoe 1979). Whitten (in Spratt and Presidente 1981) found that 71% of *T. vulpecula* examined near Taranaki in NZ were infected with *F. hepatica*.

Trichosurus vulpecula infected naturally with *F. hepatica* may show clinical and gross pathological signs consistent with migration through the hepatic parenchyma (Whittington 1982). Clinical signs may include depression, anorexia, loss of subcutaneous fat, muscle wastage and subcutaneous oedema around the ventral thorax and abdomen (Whittington 1982). Liver lesions associated with chronic fascioliasis included white fibrous tracks indicating the migrational path of the fluke in the hepatic parenchyma, as well as dilatation, thickening and fibrosis of the common bile duct (Whittington 1982).

Experimental infection of three *T. vulpecula* with 50 metacercariae produced chronic fascioliasis with severe anaemia (Boray 1969). Normal eggs of *F. hepatica* were passed in the faeces and, following embryonation, were infective for snails. Presidente (in Beveridge 1978) reported that *T. vulpecula* was highly susceptible to infection with *F. hepatica* and showed little or no resistance to reinfection.

Natural infection by *F. hepatica* has not been recorded in *T. caninus*, although experimental infection produces mature adult flukes with eggs being passed in the faeces (Presidente in Beveridge 1978; Spratt *et al*. 1991). Despite the time spent on the ground by this species (Kavanagh 1984), its habitat in tall wet open and closed forests and rainforests on the eastern coast of Australia (Owen 1964; Owen and Thompson 1965; How 1972, 1976, 1978; Lindenmayer *et al*. 1990) is not generally shared with sheep and cattle.

Cestoda (Tapeworms)

Anoplocephalidae

Only one species of anoplocephalid cestode, *Bertiella trichosuri*, has been recorded from

T. vulpecula in Australia (Green and Munday 1975; Beveridge 1976; Dunsmore 1976; O'Callaghan and Moore 1986) and NZ (Pracy and Kean 1968; Khalil 1970; Clark 1977; Warburton 1983). It has also been collected from *T. caninus* in Australia (Presidente *et al.* 1982).

Pracy and Kean (1968) discussed the possibility of transmission of *B. trichosuri* via the parasitic mites *Atellana papilio* and *Trichosurolaelaps crassipes*, which commonly inhabit the pelage of *T. vulpecula* and may be ingested by *T. vulpecula* during grooming. Assessment of the morphology of the mouthparts of *A. papilio* and *T. crassipes* indicates that this speculation may be invalid. These mites have crushing mouthparts (Viggers and Spratt, personal observations) and would probably damage the eggs of *B. trichosuri* during ingestion. The ingestion of undamaged eggs by the mites is unlikely, hence it is improbable that they would be intermediate hosts for *B. trichosuri*.

One of us (D.M.S.) observed advanced development of the eggs of *B. trichosuri* to the cysticercoid stage in soil-dwelling oribatid mites, *Zygoribatula* sp. (originating from Snowy Plains, NSW: 36°09'S, 148°33'E) and *Scheloribates* sp. B (originating from Kemps Creek, NSW; 33°53'S, 150°48'E) (Spratt, unpublished data). On the basis of these findings, Dunsmore (1976) suggested that, in areas where *T. vulpecula* was known to feed on the ground, the species may be more likely to become infected with *B. trichosuri*.

Khalil (1970) first described *B. trichosuri* from *T. vulpecula* in the northern tawa–podocarp forests of the North Island of NZ. Pracy and Kean (1968) reported that dead and emaciated animals from Taranaki in NZ were heavily infected with *B. trichosuri* and that the distribution of infected animals was discontinuous. Intensity (Margolis *et al.* 1982) of infestation was not given. A high prevalence of infection was found in animals inhabiting the ecotone between tea-tree forest and improved pasture. However, *T. vulpecula* captured in contiguous native forest were not infected with *B. trichosuri* (Pracy and Kean 1968). Analysis of the stomach contents revealed that the diet of animals from the forest–pasture ecotone consisted of up to 25% grass, whereas forest-dwelling animals ate only leaves (Pracy and Kean 1968). Pracy and Kean (1968) suggested that differences in feeding patterns between these populations may have influenced their exposure to intermediate hosts of *B. trichosuri*. Conversely, Burtton (1975) detected *B. trichosuri* in 27·3% of *T. vulpecula* inhabiting exotic pine forest *(Pinus radiata)* near Taupo on the North Island of NZ. The fact that these animals were unlikely to come in contact with pasture suggests that grazing of pasture may not be important in the life cycle of *B. trichosuri*.

In NZ, Clark (1977) reported that 27·5% of male *T. vulpecula* and 29·2% of females were infected with *B. trichosuri* (mean intensity of 13·4 worms per animal). Body weights of these animals were 12% lower than those of uninfected animals. The author suggested that young and/or emaciated animals, as well as females without pouch young, were more susceptible to infestation (Clark 1977). However, the age of animals was not determined and no other factors predisposing to infection were recorded, hence the relationship between age, weight, reproductive status and infestation with *B. trichosuri* cannot be assessed.

Warburton (1983) studied *T. vulpecula* inhabiting steep farmland and vegetated gullies in the South Island of NZ. He reported 41% prevalence of infection with *B. trichosuri* with occurrence most frequent in animals one year old (Warbuton 1983). Burdens of *B. trichosuri* were generally low (mean intensity 3·5 range 1–18), with few associated pathological changes. Warburton (1983) suggested that the lower prevalence of infection in older animals may be associated with (1) the expulsion of the parasites as the animals matured, or (2) a failure of animals to survive to adulthood. He also found that female animals with burdens of five or more adult parasites had lower mesenteric fat reserves and exhibited reduced levels of fecundity. However, sampling was terminated prior to the completion of the breeding season and may not have indicated true levels of fecundity. In addition, there was no evidence to show that relationships between parasite burdens and population measures were causative.

Presidente *et al.* (1982) recovered *B. trichosuri* from *T. caninus* at Clouds Creek in north-eastern NSW. They subjectively classified the trapping areas into two categories: (1) tall open

or closed forest, which were considered to be the preferred habitat for the species, and (2) peripheral habitat, which was any area outside preferred habitat, in exotic pine plantation, grazed woodland or open forest (Barnett *et al.* 1979*b*). Presidente *et al.* (1982) found that the prevalence of *B. trichosuri* was higher in *T. caninus* from peripheral habitat than in animals occurring in preferred habitat. However, these burdens were relatively light in animals from both habitats and there was no associated pathological changes (Presidente *et al.* 1982). The higher burdens detected in animals from peripheral habitat may have been influenced by a number of factors, including (1) differences in the diets of the animals, which may affect the probability of infection, (2) increased movement by animals in peripheral habitat, which may increase the chance of exposure to parasites, and (3) differences in the survival of free-living parasitic stages and intermediate hosts in different habitats (Presidente *et al.* 1982).

Spratt (unpublished data) found *B. trichosuri* in two of five *T. vulpecula* examined from Icena, Tas. (40°58'S, 148°30'E), in three of five *T. vulpecula* examined from Cape Gantheaume, Kangaroo Island, SA, and in one of one, two of two and four of five *T. vulpecula* examined from Bondo, Timbillica and Buckenboura (35°42'S, 150°02'E) State Forests, respectively, in south-eastern NSW.

Nematoda

A variety of nematode species have been reported from *T. caninus* and *T. vulpecula* (Beveridge 1978; Presidente 1982, 1984; Presidente *et al.* 1982; Spratt *et al.* 1991). Some of these presumably have direct life cycles, others indirect via invertebrate intermediate hosts, but no cycle has been elucidated in its entirety (Presidente 1982, 1984; Presidente *et al.* 1982; Spratt 1986). Several trichostrongyloid species occurring in *T. vulpecula* have been acquired accidentally from sheep and cattle (Bearup and Bolliger 1949; Sweatman and Williams 1962; O'Callaghan and Moore 1986). None of these has been reported in *T. caninus* (Spratt *et al.* 1991), probably reflecting a major difference in habitat use between these two host species.

Rhabditoidea : Strongyloididae

Parastrongyloides trichosuri. Species of the genus *Parastrongyloides* have both parasitic and free-living generations each with adult male and female worms. Eggs laid by older free-living females are fully embryonated or retained *in utero* until they hatch (Mackerras 1959). Some larvae may develop into a second generation of free-living adults. Others are destined to become infective larvae and, after infection, develop into parasitic males and females. Parasitic females lay eggs that pass out, in faeces, in various stages of development up to fully embryonated and newly hatched (Mackerras 1959). These either (1) develop into free-living male and female worms that eventually produce infective larvae, or (2) develop directly into infective larvae.

Parastongyloides trichosuri inhabits the proximal region of the small intestine, particularly the upper ileum (Mackerras 1959; Presidente *et al.* 1982). Low numbers of this parasite produce only mild inflammation in the lamina propria (Presidente *et al.* 1982).

Parastongyloides trichosuri has been found in populations of *T. vulpecula* in mainland Australia (Mackerras 1959; Presidente *et al.* 1982), Tasmania (Spratt, unpublished data) and NZ (Burtton 1975), but not in animals on Kangaroo Island (O'Callaghan and Moore 1986). Spratt (unpublished data) found *P. trichosuri* in three of five, two of two and two of five *T. vulpecula* from Icena in Tas., and Timbillica and Buckenboura State Forests in south-eastern coastal NSW, respectively. *P. trichosuri* also was collected from a population of *T. caninus* at Clouds Creek, where it was detected at low levels in all animals (n = 57) from both preferred and peripheral habitats and in all *T. vulpecula* (n = 3) in an area sympatric with *T. caninus* from peripheral habitat (Presidente *et al.* 1982). *P. trichosuri* has not been recorded from hosts other than *T. vulpecula* and *T. caninus* (Spratt *et al.* 1991).

Strongyloides sp. A species of *Strongyloides* has been recorded from *T. vulpecula* near Sydney (Gordon and Somerville 1958). These authors suggested that sheep are generally

primary hosts of parasites of the genus *Strongyloides* and were probably the original source of infection for *T. vulpecula*. No additional records of *Strongyloides* sp. in *Trichosurus* spp. have been published.

Trichostrongyloidea : Herpetostrongylidae

Paraustrostrongylus trichosuri. *P. trichosuri* is usually found coiled around the villi of the proximal small intestine (Mawson 1973; Smales and Mawson 1978; Presidente 1984) and is known exclusively from *T. caninus* and *T. vulpecula*, although it appears to be rare in NZ populations of *T. vulpecula* (Spratt *et al.* 1991). Clark (unpublished data) found a heavy burden of parasites (9600) in one emaciated individual found dead in the Orongorongo Valley.

Infection of *T. vulpecula* with *P. trichosuri* was detected on Kangaroo Island, by Smales and Mawson (1978), but not by O'Callaghan and Moore (1986) or Spratt (unpublished data) in subsequent studies. O'Callaghan and Moore (1986) considered that *P. trichosuri* may have been replaced by *Trichostrongylus* spp. from domestic livestock.

Paraustrostrongylus trichosuri was detected in all *T. caninus* (n = 57) and *T. vulpecula* (n = 3) examined at Clouds Creek (Presidente *et al.* 1982). The highest burdens of parasites were recorded from *T. caninus* in peripheral habitat. This may be related (1) to increased movement and contact between individuals in these areas, (2) to dietary difference, which may influence the probability of exposure to parasites, and (3) to the social status of individuals. Esch *et al.* (1975) found that animals of lower social status tended to carry heavier burdens of parasites. The lower burdens detected in adult animals may be due (1) to acquired immunity, or (2) to differences in the diet between age groups (Presidente *et al.* 1982). Subadult males supported burdens that were significantly heavier than those detected in subadult females. Male *T. caninus* disperse when they are subadults after a long period within the maternal home range (How 1972, 1981). Hence, the increased prevalence of *P. trichosuri* in this age cohort may be associated with the process of dispersal, rather than the age of the animals (Presidente *et al.* 1982). Presidente *et al.* (1982) also found that adult female *T. caninus* supported heavier burdens than did adult males, possibly because of the influence of hormones or reproductive status, although no evidence was provided. Spratt (unpublished data) found *P. trichosuri* in two of five, two of two, and two of five *T. vulpecula* from Icena in Tas. and Buckenboura and Timbillica State Forests in south-eastern coastal NSW, respectively.

Dromaeostrongylidae

Peramelistrongylus sp., *Profilarinema hemsleyi* and *Profilarinema* sp. have been detected in *T. vulpecula* (Durette-Desset and Beveridge 1981; Spratt *et al.* 1991). However, there is only limited information on the geographic distribution and abundance of these parasites in this host. Durette-Desset and Beveridge (1981) described *P. hemsleyi* from the stomach and small intestine of *T. vulpecula* from WA. *Peramelistrongylus skedastos* typically occurs in bandicoots and small dasyurids and there is an undescribed species known from the rufous rat kangaroo, *Aepyprymnus rufescens*.

Filarinema trichosuri is known only from female worms from the oesophagus of *T. caninus* in the Gosford region of NSW (Johnston and Mawson 1939*a*). All other species of the genus *Filarinema* occur in the stomach and small intestine of macropodoid marsupials and *F. trichosuri* is probably a *nomen nudum* that fails to satisfy the technical rules for availability for Zoological Nomenclature.

Trichostrongylidae

Trichostrongylus colubriformis is a common parasite of sheep (Blood and Radostits 1989). It has been identified in populations of *T. vulpecula* that inhabit areas adjacent to agricultural land in Australia (e.g. Kangaroo Island, SA: O'Callaghan and Moore 1986) and NZ (Sweatman and Williams 1962; Bowie and Bennett 1983). This parasite has not been detected in forest

populations of *T. vulpecula* in NZ (Bowie and Bennett 1983) nor in *T. caninus* in Australia (Spratt *et al.* 1991). Infection probably is acquired when possums graze pasture that is contaminated by the faeces of infected sheep (Bowie and Bennett 1983). Although the intensity of infection by *T. colubriformis* in possums is variable (range 2–576 worms per individual), pathogenicity appears to be minimal even when numbers of the parasite are moderately high (Bowie and Bennett 1983). However, Bearup and Bolliger (1949) reported clinical signs of diarrhoea, dehydration and loss of appetite in one *T. vulpecula* in Australia that was found to be carrying high numbers (>5000) of *T. colubriformis* and some *Trichostongylus rugatus*. These parasites also were detected in 22 *T. vulpecula* being held in captivity at this time (Bearup and Bolliger 1949).

Trichostongylus axei, *T. rugatus* (Bearup and Bolliger 1949; Smales and Mawson 1978), *T. retortaeformis* (Burtton 1975) and *T. vitrinus* (Gordon and Somerville 1958) are other *Trichostrongylus* spp., commonly found in sheep and cattle, that also have been recorded from *T. vulpecula*. Burtton (1975) detected *T. retortaeformis* in 68·2% of *T. vulpecula* from pine forests near Taranaki on the North Island of NZ. Other parasites commonly found in domestic livestock that have been reported from *T. vulpecula* near Sydney are *Cooperia curticei* and a species of *Nematodirus* (Gordon and Somerville 1958).

There are no records of infection of *T. caninus* with parasites from sheep or cattle. This may be because *T. caninus* inhabits tall open and closed sclerophyll forests and temperate rainforest along the east coast of Australia. It is usually a forest-dwelling species (Owen 1964; Owen and Thomson 1965; How 1972; Lindenmayer *et al.* 1990) and may not come into contact with pasture grazed by domestic livestock.

Metastrongyloidea : Angiostrongylidae

Lungworms have not been recorded from *T. vulpecula* on Kangaroo Island (Smales and Mawson 1978; O'Callaghan and Moore 1986; Spratt, unpublished data).

Marsupostrongylus minesi. Spratt (1979) described *Marsupostrongylus minesi* from sections of the terminal bronchioles of the lungs of *Trichosurus* spp. (probably *T. caninus)* from south-eastern Qld. In addition, lung tissue from both *T. caninus* and *T. vulpecula* in north-eastern NSW has been examined and *M. minesi* was found in the lungs of *T. caninus* only, indicating that *T. caninus* is a definitive host of this parasite (Presidente *et al.* 1982). Presidente *et al.* (1982) reported minimal inflammatory reaction around mature worms in alveolar sacs. However, lungworm larvae were associated with focal interstitial thickening, smooth-muscle hyperplasia and occasional granulomatous lesions. Subsequently, Bellamy (1993) reported a number of clinical cases in populations of *T. vulpecula* near Sydney, NSW, where *M. minesi* was diagnosed as the cause of respiratory distress and cyanosis. At post-mortem examination, multiple pale foci were found in the lungs due to verminous pneumonia attributed to *M. minesi* (Bellamy 1993).

Marsupostrongylus pseudominesi. *M. pseudominesi* is known only from the lower bronchi, bronchioles and dilated alveoli of the northen subspecies of *T. vulpecula*, *T. v. johnstonii* (Spratt 1984). The presence of this parasite is associated with mild pulmonary inflammation.

Marsupostrongylus longilarvatus. *M. longilarvatus* has been recorded from *T. vulpecula* from Nadgee State Forest, NSW (Spratt 1984). This parasite has the most catholic host range of any species in the genus and has been recorded from macropodid, pseudocheirid and phascolarctid marsupials (Spratt 1979, 1984).

Filostrongylus tridendriticus. *F. tridendriticus* is known only from *T. vulpecula* in Tas. (Spratt 1984), although the precise site of infection in the lungs is unknown.

Oxyuroidea : Oxyuridae

Adelonema trichosuri. Female oxyurids from *T. vulpecula* in Qld were first described as *Syphacia trichosuri* by Johnston and Mawson (1938). The species was later redescribed from male and female specimens as *Adelonema trichosuri* by Mawson (1978). This parasite may occur in large numbers in the caecum of *T. vulpecula* and *T. caninus*, with few associated pathological changes (Presidente 1982). *A. trichosuri* occurs more commonly in *T. caninus*, although it has also been detected in *T. vulpecula* on Kangaroo Island, SA (Smales and Mawson 1978; O'Callaghan and Moore 1986), and in one of five and one of one *T. vulpecula* in Timbillica and Bondo State Forests, south-eastern NSW (Spratt, unpublished data), respectively.

At Clouds Creek there was a higher prevalence of this parasite in *T. caninus* from peripheral habitat compared with those from preferred habitat (Presidente *et al.* 1982). Burdens of *A. trichosuri* in *T. caninus* generally were low, but moderate-to-heavy burdens also were detected with no associated pathological changes (Presidente *et al.* 1982).

Nematodes of pythons: Ascaridoidea : Ascarididae

Ophidascaris (=Amplicaecum) robertsi. The definitive host of *Ophidascaris robertsi* is the carpet python, *Morelia spilota variegata*, but a broad spectrum of rodents, reptiles and marsupials may serve as intermediate hosts of this parasite (Sprent 1963*a*, 1963*b*; Spratt *et al.* 1991). Third-stage larvae of *O. robertsi* (up to 34 mm long) occur in many tissues and organs in both the pleural and peritoneal cavities (Sprent 1963*b*; Spratt *et al.* 1991). In the intermediate host, it takes at least four weeks for the parasite eggs to develop to third-stage larvae, which are infective for the carpet python (Sprent 1963*b*). The development of third-stage larvae in *T. caninus* is faster than in some other intermediate hosts, such as the bush rat, *Rattus fuscipes* (Sprent 1963*b*). If the intermediate host has been infected for four or more weeks and is then eaten by a carpet python, ingested third-stage larvae will moult and complete their development to adults in the wall of the stomach and oesophagus of the definitive host (Sprent 1963*b*).

Ophidascaris robertsi has been found in the liver of *T. vulpecula* and *T. caninus* in north-eastern and south-eastern NSW (Sprent 1963*b*; Presidente 1978, 1984; Presidente *et al.* 1982). A total of 54% of *T. caninus* examined at Clouds Creek had lesions associated with infection by *O. robertsi* (Presidente *et al.* 1982). However, a female with 216 *O. robertsi* remained in good condition (assessed from internal fat depots) and was reproductively active, despite extensive liver damage (Presidente *et al.* 1982). Some *T. caninus* from Clouds Creek harboured up to 400 larvae of *O. robertsi;* these comprised up to 30–40% of the total weight of the liver and lesions occured just beneath the capsular surface of the liver. Larvae caused extensive pathological changes in hepatic parenchyma, including focal cholangitis and dilatation and fibrosis of bile ducts that contained third-stage larvae (Sprent 1963*b*; Presidente *et al.* 1982).

Ophidascaris robertsi occurred in all three *T. vulpecula* examined at Clouds Creek, NSW (Presidente *et al.* 1982), with liver lesions similar to those described for *T. caninus*.

Spiruroidea (Gongylonematidae and Spirocercidae)

Protospirura marsupialis and an undescribed species of *Gongylonema* have been reported from the oesophageal and gastric mucosae of *T. vulpecula* from Qld and NSW (Johnston and Mawson 1939*b*; Spratt *et al.* 1991). *Gongylonema* sp. was found in 16 of 21, four of five and one of five *T. vulpecula* from Tas., Buckenboura State Forest in south-eastern NSW and Kangaroo Island, respectively (Spratt, unpublished data). Presidente *et al.* (1982) recorded no spirurid nematodes in possums at Clouds Creek, NSW, and these parasites have not been reported from animals in NZ.

Filarioidea : Onchocercidae

Breinlia trichosuri. *B. trichosuri* was described from *T. vulpecula* in Qld; microfilariae are unsheathed and occur in the peripheral blood (Breinl 1913; Spratt and Varughese 1975). Adult

parasites are found in the peritoneal cavity among the mesentery (Mackerras 1962; Spratt and Varughese 1975; Chabaud and Bain 1976; Beveridge in Presidente 1984). One animal from which 45 *B. trichosuri* were collected had diffuse peritonitis, indicating that heavy burdens of this parasite may be pathogenic (Beveridge in Presidente 1984). Bolliger (1951) detected microfilariae in 20% of *T. vulpecula* examined from Moss Vale, near Sydney, but no adult parasites were found. No microfilariae were detected in urban animals from Sydney (Bolliger 1951). Spratt (unpublished data) recovered *B. trichosuri* from one of one and one of five *T. vulpecula* from Nadgee and Mogo State Forests, respectively, in south-eastern NSW.

This parasite also has been detected in *T. caninus* in NSW (Johnston and Mawson 1938) but has not been recorded from *T. vulpecula* in NZ (Spratt *et al.* 1991; Clark, unpublished data).

Sprattia (= Dipetalonema) venacavincola. *S. venacavincola* was first described from the caudal vena cava and the hepatic veins of *T. caninus* (Spratt and Varughese 1975). Presidente *et al.* (1982) failed to find adults of *S. venacavincola* in *T. caninus* at Clouds Creek, NSW, but detected sheathed microfilariae in blood smears from 25% of *T. caninus* and reported nodules in the spleen, which represented granulomatous reactions to the presence of sequestered microfilariae (Presidente *et al.* 1982). Life-cycle studies of this species have been attempted with wild mosquitoes (Spratt and Varughese 1975). *Culex (Culex) fatigans* were attracted to infected *T. caninus* but did not attempt to feed. *Aedes (Ochlerotatus) vigilax* fed to engorgement on an infected animal, but dissection of mosquitoes between 16 h and five days after the blood meal revealed dead microfilaria in the gut, suggesting that *A. vigilax* is not a suitable intermediate host of *S. venacavincola*. There are no confirmed records of *S. venacavincola* from *T. vulpecula* in Australia or NZ (Spratt *et al.* 1991). However, Presidente *et al.* (1982) recorded hepatic vasculitis in six of 57 *T. caninus* and one of three *T. vulpecula* at Clouds Creek, NSW, which may have been associated with infection by *S. venacavincola*.

Cercopithifilaria johnstoni. *C. johnstoni* occurs in the native rodents *Rattus fuscipes*, *R. lutreolus* and *Uromys caudimaculatus* (Muridae), the marsupial bandicoots *Perameles gunnii*, *P. nasuta*, *Isoodon macrourus* and *I. obesulus* (Peramelidae), the glider *Petauroides volans* (Petauridae) and the Tasmanian devil, *Sarcophilus harrisii* (Dasyuridae) (Spratt and Haycock 1988). Recently, this catholic filarioid species which utilises ixodid ticks as intermediate host (Spratt and Haycock, 1982) has been found in *Trichosurus vulpecula* (Phalangeridae) in Tasmania (Spratt, unpublished data).

Ectoparasites

A number of mites, ticks and fleas, but no lice, has been collected from *T. vulpecula* and *T. caninus* in Australia (Domrow 1961, 1962, 1974, 1978, 1987, 1991, 1992; Roberts 1970; Dunnet and Mardon 1974; Doube 1974, 1975; Emerson and Price 1981; Presidente *et al.* 1982). Some species of mites but no fleas or ticks have been recorded from *T. vulpecula* in NZ (Sweatman 1962; Mitchell and Strandtmann 1964; Clark, unpublished data). Nineteen species of mites have been found on *T. caninus* and *T. vulpecula*.

Engorging ticks and tick-bite lesions are found commonly around the neck, beneath the mandible, on the face and around the scrotum or pouch of the host (Presidente 1984). Some tick species may cause irritation, anaemia, oedema at the site of attachment, toxaemia and paralysis in host animals (Roberts 1970; Presidente 1984). In some regions, 'rump wear' has been found on *T. caninus* and *T. vulpecula* (Presidente 1982, 1984; Presidente *et al.* 1982; Hemsley and Canfield 1993) and may be associated with an allergic reaction to mite infestation (Presidente *et al.* 1982). Although mites and fleas have been found on some affected animals, the presence of ectoparasites has not always been associated with the condition (Hemsley and Canfield 1993). The cause of rump wear is probably multifactorial and contributing factors may include stress, mites and/or fleas, *Trichophyton* spp., bacterial infection, debilitation and underlying disease (Hemsley and Canfield 1993). The ectoparasite species known from *Trichosurus* spp. and aspects of their biology and ecology are outlined below.

Arachnida : Acari (Ticks and Mites)

Ixodoidea : Ixodidae (Ticks)

Ixodes spp. Species of *Ixodes* that have been found on *T. vulpecula* in eastern Australia include *I. holocyclus, I. tasmani, I. trichosuri, I. cornuatus, I. hirsti* and *I. fecialis* (Roberts 1970). *I. holocyclus, I. tasmani, I. hirsti* and *I. trichosuri* have been recorded from *T. caninus* (Roberts 1970).

Ixodes holocyclus. The long-nosed bandicoot, *Perameles nasuta*, and short-nosed bandicoots, *Isoodon macrourus* and *I. obesulus*, appear to be the most common hosts for *I. holocyclus*. However, this tick has been detected on a range of other native mammal species including *T. vulpecula* and *T. caninus* (Roberts 1970; Doube 1975). *T. caninus* may carry large numbers of ticks but appears to be resistant to the toxin (Doube 1975). Doube (1975) considered that, although *T. caninus* acquires moderate numbers of *I. holocyclus* because of its ground-dwelling behaviour in south-eastern Qld, *T. caninus* was too uncommon to be a major host resource for the tick. However, the transmission of disease organisms depends on the distribution and abundance of ticks within communities of animals, and because different stages of the tick attach and engorge on different host species, most native hosts probably are important in the life cycle of *I. holocyclus* (see Spratt and Haycock 1988). Doube (1974) demonstrated experimentally in the laboratory that all three stages of *I. holocyclus* will feed to engorgement on both *T. caninus* and *T. vulpecula*.

Ixodes holocyclus was the most commonly detected tick on *T. caninus* at Clouds Creek and the intensity of infestation was greatest on animals in peripheral habitat (Presidente *et al.* 1982). Individuals of *T. vulpecula* sympatric with *T. caninus* in peripheral habitat at Clouds Creek were not infested with *I. holocyclus* (Presidente *et al.* 1982). This supports Doube's (1974) evidence of resistance in *T. vulpecula* to the establishment of *I. holocyclus*. Doube (1974) suggested that the probability of infestation by ticks may be related to foraging behaviour; however, no supporting evidence was offered.

Ixodes holocyclus is a vector of north Qld tick typhus, caused by *Rickettsia australis* (Campbell and Domrow 1974), and Fenner (1946) concluded on serological evidence that *T. vulpecula johnstonii* was one of five probable native mammal reservoirs of infection.

Ixodes tasmani. *I. tasmani* has a wide host and geographic distribution in Australia (Roberts 1970) and has been reported from *T. vulpecula* in Tas., Vic., NSW, ACT, WA, Qld and on Kangaroo Island, SA (Nuttall 1916; Fenner 1946; Roberts 1960, 1964, 1970; Cambell and Domrow 1974; O'Callaghan and Moore 1986). Although *I. tasmani* was collected infrequently from *T. caninus* at Clouds Creek it was more prevalent on animals found in peripheral habitat (Presidente *et al.* 1982).

Ixodes trichosuri. *I. trichosuri* has a geographic distribution restricted to south-eastern NSW, Vic. and Tas. (Roberts 1964, 1970). Described originally from *T. vulpecula* in Sydney, it has since been found on several native marsupial and murid mammals, although adult ticks have been found exclusively on *Trichosurus* spp. (Roberts 1970). There is a single published record of *I. trichosuri* on *T. caninus* in NSW (Kemp in Presidente 1984).

Ixodes cornuatus. *I. cornuatus* is closely related to *I. holocyclus* but is restricted geographically to south-eastern NSW, eastern Vic. and Tas. and has been recorded from far fewer host species (Roberts 1964, 1970). Doubt remains concerning the distinction of these two species on mainland Australia. It has been suggested that *I. cornuatus* was the cause of two cases of tick paralysis in domestic animals near Orbost, Vic. (Roberts 1970), although *I. holocyclus* was also retrieved from these hosts (Arundel in Mason *et al.* 1974). In Tas., where

I. holocyclus does not occur, there also has been a small number of reported cases of paralysis in domestic pets, which were associated with the presence of engorged *I. cornuatus* (Mason *et al.* 1974).

Ixodes hirsti. *I. hirsti* has a southern distribution (south-eastern NSW, Vic., south-eastern SA, Tas. and WA) and is known from two rat species and a spectrum of marsupial hosts, including *T. vulpecula* and *T. caninus* (Roberts 1964, 1970).

Ixodes fecialis. *I. fecialis* has a wide distribution from east to west in the southern half of Australia, including Tas. but has been recorded only once on *Trichosurus* spp. (Roberts 1970).

Haemaphysalis spp. In Australia, ticks of the genus *Haemaphysalis* occur predominantly in Qld, NT and north-eastern NSW (Roberts 1963, 1970). Species that have been found on *T. vulpecula* in Australia include *H. bremneri*, *H. bancrofti*, *H. ratti* and *H. humerosa* (Smith 1940; Roberts 1963, 1970), although the latter record (Smith 1940) may be doubtful, as *H. bremneri* often has been confused with *H. humerosa* (Roberts 1963). No *Haemaphysalis* spp. have been recorded from *T. caninus* (Roberts 1970; Presidente 1984).

Haemaphysalis bremneri. *T. vulpecula* is a primary host of *H. bremneri*, but this tick species has been recovered from other unrelated host animal species (Roberts 1963, 1970). *H. bremneri* has been recorded from *T. vulpecula* in south-eastern Qld (Roberts 1963).

Haemaphysalis bancrofti. This tick species occurs primarily on members of the Macropodidae, especially wallabies, but also has been recorded from *T. vulpecula* in north-eastern NSW and south-eastern Qld (Domrow and Smith 1956; Roberts 1963, 1970).

Haemaphysalis ratti. *H. ratti* has a wide distribution on a range of mammalian hosts in northern, western and southern Australia, but has been reported from *T. vulpecula* only in south-eastern Qld (Roberts 1963, 1970). A single record of *H. ratti* from 'a large opossum' from Bathurst Island in NT (Roberts 1963) may have been from the northern race, *T. vulpecula arnhemensis*.

Haemaphysalis humerosa. *H. humerosa* is most commonly found on the northern brown bandicoot, *Isoodon macrourus*, but has also been collected from a range of other marsupial and eutherian mammal and bird hosts including *T. vulpecula* in south-eastern and central Qld (Smith and Derrick 1940*a*, 1940*b*; Roberts 1963, 1970). *H. humerosa* has been identified as the major vector of the aetiological agent of Qld tick typhus, *Rickettsia australis* (see Fenner 1946). Fenner (1946) concluded on serological evidence that *T. vulpecula johnstonii* probably was a reservoir of *R. australis*.

Ixodoidea : Argasidae

Ornithodoros spp. Only one argasid tick species, *Ornithodoros macmillani*, has been recorded from *T. vulpecula* in Qld (Kemp in Presidente 1978). *O. macmillani* occurs primarily in the nests of the galah, *Cacatua roseicapilla* (Roberts 1970).

Mesostigmata (Mites) : Laelapidae

Laelapid mites recorded from *T. vulpecula* in Australia include *Trichosurolaelaps crassipes* (see Domrow 1972, 1987), *T. dixous* (see Domrow 1972, 1987), *Haemolaelaps penelope*, *H. sisyphus* (see Domrow 1979, 1980, 1987) and *Laelapsella humi*. In NZ, *T. crassipes* is the only dermanyssid mite recorded from *T. vulpecula* (Sweatman 1962; Mitchell and Strandtmann 1964; Bowie and Bennett 1983; Clark 1993). Only *T. crassipes*, *T. dixous* and *H. penelope* have been recorded from *T. caninus* (Presidente *et al.* 1982; Domrow 1987).

Trichosurolaelaps crassipes. *T. crassipes* is the most commonly recorded species of mite from *T. caninus* and *T. vulpecula* in eastern Australia (Domrow 1966, 1972, 1979; Presidente *et al*. 1982; Presidente 1984). It also has been recorded from *T. vulpecula* in SA (Domrow 1961). *T. crassipes* is a shiny, highly pigmented, haematophagus mite that lives on the surface of the skin. It has been postulated that when present in large numbers this species may cause irritation (Domrow 1979; Presidente 1982, 1984). Digestion of skin from *T. vulpecula* with rump wear revealed high numbers of *T. crassipes* (Presidente 1978). However, in NZ, this mite has a uniform distribution across the pelage of *T. vulpecula* and the extent to which it feeds on the rump is unknown (Clark 1993).

Presidente *et al*. (1982) found *T. crassipes* commonly on *T. caninus* at Clouds Creek, NSW, and on *T. vulpecula* that were sympatric with *T. caninus*. *T. crassipes* has been reported from *T. vulpecula* in NZ (Bowie and Bennett 1983; Clark, unpublished data). In the Orongorongo Valley, NZ, *T. crassipes* occurred over the entire body of *T. vulpecula*, although it was concentrated predominantly on the ventral surfaces of the limbs and on the rump above the tail (Clark 1993).

Trichosurolaelaps dixous (=dixoa). *T. dixous* has been reported only from *T. caninus* in south-eastern Qld and north-eastern NSW (Domrow 1972, 1987; Presidente *et al*. 1982). There are no other host records for this species.

Haemolaelaps penelope. This mite has been recorded only from *T. caninus* in south-eastern Qld and north-eastern NSW (Domrow 1964, 1979, 1987; Presidente *et al*. 1982).

Haemolaelaps sisyphus. *H. sisyphus* is known only from *T. vulpecula* in south-eastern NSW (Domrow 1980, 1987).

Laelapsella humi. *L. humi* was first described from mutton birds, *Puffinus tenuirostris*, by Womersley (1955) and has since been recorded from *T. vulpecula* only in Vic. (Domrow 1973).

Rhinonyssidae

Ornithonyssus sp. Ear mites of the genus *Ornithonyssus* were collected from five *T. vulpecula* on Kangaroo Island, SA (O'Callaghan and Moore 1986).

Sarcoptiformes : Atopomelidae

Atellana papilio. Very few astigmatid mites have been reported in Australia. *A. papilio* was first described from *T. vulpecula* in south-eastern Qld (Domrow 1958) and also is known from this host in south-eastern NSW and Tas. It is most commonly detected on the thighs and rump of the host (Domrow 1958). *A. papilio* was found in low numbers on *T. caninus* at Clouds Creek, NSW (Presidente *et al*. 1982; Domrow 1992), and has been recorded from *T. vulpecula* in NZ (see below). This species of mite has not been reported from other hosts (Domrow 1992).

Petrogalochirus dycei. *P. dycei* was found in low numbers on *T. caninus* at Clouds Creek, NSW (Presidente *et al*. 1982), and is known from *T. vulpecula* in south-eastern NSW and Vic. (Domrow 1992).

Both *A. papilio* and *P. dycei* have been recorded from *T. vulpecula* in NZ (Sweatman 1962; Bowie and Bennett 1983; Clark 1993). In a detailed study of the fur-inhabiting mites of seven *T. vulpecula* from Taranaki in NZ, Clark (1993) recorded *A. papilio*, *P. dycei* and *Murichirus* sp. (see below) in relative total abundances of 17%, 43% and 34% respectively (*T. crassipes* comprised the remaining 6% of all mites collected). The distribution of some mites across the pelts of the animals was not uniform (Clark 1993). Large numbers of eggs and mites of *A. papilio* were found in the rump fur of *T. vulpecula*, whereas *Murichirus* sp. were collected

predominantly from the forepart of the animal and *P. dycei* was detected in the fur of the dorsum (Clark 1993).

Murichirus sp. Domrow (1992) described *Murichirus anabiotus* from the common ringtail possum, *Pseudocheirus peregrinus*, from Dartmouth, Vic., but, after identifying a *Murichirus* sp. from *T. vulpecula* in NZ (see Clark 1993), he considered that the original material from *P. peregrinus* may have been contaminants. *Murichirus* sp. has recently been found on *T. caninus* at a number of sites throughout the geographic range of the host (Viggers, unpublished data).

Sarcoptiformes : Echimyopodidae

Marsupiopus trichosuri. *M. trichosuri* is known exclusively from *T. vulpecula* in ACT (Fain 1968; Domrow 1992).

Sarcoptiformes : Sarcoptidae

Notoedres muris is known from a range of murid hosts from all states of Australia as well as from *T. vulpecula* in Vic. (Domrow 1992).

Prostigmata : Trombidiformes : Trombiculidae

A number of trombiculid mites have been collected from *T. caninus* and *T. vulpecula*. These are *Ascoschoengastia rattus* (see Domrow 1974, 1978), *Eutrombicula hirsti*, *Guntheria lappacea*, (see Domrow 1978), *G. kallipygos* (see Domrow 1978), *G. pseudomys*, (see Domrow 1978), *G. trichosuri*, (see Domrow 1978), *G. shieldsi*, *G. peregrina*, *Neotrombicula novaehollandia* (see Domrow 1974) and *Trombicula quadriensis* (see Domrow 1978). These larval mites penetrate the epidermis of the skin and may cause localised inflammation (Sweatman 1962).

Eutrombicula hirsti. This mite is known from several marsupial hosts, including *T. vulpecula* from south-eastern Qld (Domrow and Lester 1985).

Neotrombicula novaehollandiae. *N. novaehollandiae* has been recorded from *T. vulpecula* in Tas. (Green and Munday 1971) as well as from numerous native mammals and rats in Vic., NSW, south-eastern SA and Tas. (Domrow and Lester 1985).

Ascoschoengastia rattus. *A. rattus* has been recorded from the nasal passages of *T. vulpecula* from Tas. and south-eastern Qld and from *T. caninus* in south-eastern Qld (Domrow 1962, 1974, 1978). It is the most common nasal parasite of *T. vulpecula* in south-eastern NSW with several hundred mites occurring in the nasal sinuses of some individual hosts (Spratt, unpublished data). This mite is known from a number of other marsupial and murid hosts (Womersley and Heaslip 1943; Domrow and Lester 1985).

Trombicula quadriensis. *T. quadriensis* has been recorded from *T. vulpecula* in south-eastern Qld and Tas. (Domrow 1974, 1978; Domrow and Lester 1985). It also has been found on *T. caninus* at Mt Glorious in south-eastern Qld (Domrow 1978). *T. quadriensis* occurs on several other marsupial and murid hosts (Womersley and Heaslip 1943; Domrow 1978; Domrow and Lester 1985).

Guntheria peregrina. *G. peregrina* has been recorded from relatively few hosts, including *T. vulpecula* from south-eastern Qld (Domrow and Lester 1985).

Guntheria trichosuri. *G. trichosuri* has been recorded only from *T. vulpecula* in south-eastern Qld (Domrow 1960, 1978; Domrow and Lester 1985). There are no other host records

for this species. *G. trichosuri* was originally described from *T. vulpecula* from northern coastal NSW (Womersley 1939). The holotype may have been mistakenly identified, as remounted paratype specimens were later identified as *T. quadriensis* by Domrow (1978).

Guntheria shieldsi. *G. shieldsi* is known from numerous marsupial and murid hosts and also from *T. vulpecula* from coastal eastern Australia (Domrow and Lester 1985).

Guntheria pseudomys. *G. pseudomys* occurs on native rats and *T. vulpecula* from northern Qld, the type being described from the latter host (Womersley and Audy 1957; Domrow 1960; Domrow and Lester 1985).

Guntheria lappacea. *G. lappacea* has been recorded from numerous hosts in coastal eastern Australia including *T. caninus* from Canungra in Qld (Domrow 1978; Domrow and Lester 1985).

Guntheria kallipygos. This mite was first described by Gunther (1939) and occurs on many native mammal and murid hosts in coastal eastern Australia including both *T. vulpecula* in Tas. and *T. caninus* in south-eastern Qld (Domrow 1962, 1964, 1978; Domrow and Lester 1985).

Prostigmata : Leeuwenhoekiidae

Parasitic larvae of *Odontacarus* sp. have been recorded from *T. vulpecula* in Qld; adults of this genus are free-living (Domrow 1991). The larvae of *Odontocarus* sp. generally are found in clusters on any part of the body, including the head, neck, abdomen, pouch and scrotum, particularly where there is broken tissue (e.g. around a tick that is attached on the host's ear: Domrow 1962).

Siphonaptera (Fleas)

Pygiopsyllidae

The pygiopsyllid flea species that typically occur on *T. vulpecula* are *Choristopsylla ochi*, *C. tristis*, *Bibikovana hoplia*, *Acanthopsylla pavida* and *A. rothschildi rothschildi* (see Dunnet and Mardon 1974; Mardon 1977).

Choristopsylla ochi (=C. leptophallus). *C. ochi* has been reported from *T. vulpecula* throughout most of its range from WA, through SA, Tas. and Vic. and along the east coast to northern Qld (Green and Munday 1971; Dunnet and Mardon 1974). It is also known from a number of other phalangerid and petaurid marsupials (Dunnet and Mardon 1974).

Choristopsylla tristis. *C. tristis* has been recorded from *T. vulpecula* in Qld (Mardon and Allison 1978).

Bibikovana (=Pygiopsylla) hoplia. *B. hoplia* has been recorded from *T. vulpecula* in Vic., Tas. and Qld (Green and Munday 1971; Dunnet and Mardon 1974; Traub 1980). This flea also has been collected commonly from numerous other marsupial and rodent hosts (Dunnet and Mardon 1974).

Acanthopsylla pavida. *A. pavida* has been recorded from *T. vulpecula* in Atherton, Qld, and from *T. caninus* in north-eastern NSW (Dunnet and Mardon 1974; Presidente *et al*. 1982). This flea occurs on a number of other arboreal marsupial hosts.

Acanthopsylla rothschildi rothschildi. *A. rothschildi rothschildi* is widely distributed across south-eastern Australia (south-eastern Qld, NSW, ACT, Vic. and Tas.), on a large number of native mammal and rodent hosts. It has been recorded from *T. vulpecula* in Tas. and Vic. (Dunnet and Mardon 1974).

Pulicidae

Echidnophaga myrmecobii. *E. myrmecobii* was found attached to the ear margins and eyelids of *T. vulpecula* in Vic. (Presidente 1982). This species has also been recorded from *T. vulpecula* in WA, SA, Vic. and NSW (Jordan and Rothschild 1922; Roberts 1970; Dunnet and Mardon 1974).

T. vulpecula *as an accidental host*

Flea species that have been found on *T. vulpecula* as an accidental host are *Echinophaga gallinacea* (the stickfast flea), *Pulex irritans* (the human flea) and *Ctenocephalides felis* (the cat flea) (Green and Munday 1971; Dunnet and Mardon 1974).

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APPENDIX 2

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The use of tiletamine hydrochloride and zolazepam hydrochloride for sedation of the mountain brushtail possum, *Trichosurus caninus* Ogilby (Phalangeridae: Marsupialia)

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SUMMARY : A combination of tiletamine hydrochloride and zolazepam hydrochloride in a 1:1 ratio by weight was used successfully to sedate mountain brushtail possums, *Trichosurus caninus*, in the field. A standard total dose of 50 to 60 mg provided adequate sedation for the completion of a range of handling procedures. We describe the induction time, dose rate and side-effects associated with the use of tiletamine and zolazepam in *T caninus*.

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Introduction

Dissociative anaesthetic agents are frequently used for sedation of wildlife in the field (Bush *et al* 1978, 1980; Haigh *et al* 1985; Loughlin and Spraker 1989; Stirling *et al* 1989; Kreeger *et al* 1990a, b; Phelan and Green 1992). A number of studies have revealed that tiletamine hydrochloride and zolazepam hydrochloride given in a 1:1 combination by weight, has a faster induction and recovery time and fewer side-effects than other dissociative anaesthetic agents (Bush *et al* 1978, 1980). Tiletamine hydrochloride, a cyclohexamine derivative, produces cataleptoid or dissociative anaesthesia and, when used in combination with zolazepam hydrochloride (a diazepinone tranquiliser), is a safe and effective sedative or anaesthetic agent with good muscle relaxation and analgesia (Booth 1988; Van Heerden *et al* 1991). Used alone, tiletamine may cause convulsions in some species but this side-effect is reduced substantially when the drug is combined with zolazepam (Booth 1988; Van Heerden *et al* 1991). Animals sedated or anaesthetised with this drug combination retain the palpebral, corneal and swallowing reflexes, and the eyes may remain open (Bush *et al* 1978; Van Heerden *et al* 1991).

This study was completed as part of a larger project to investigate the parasitology, blood chemistry, clinical variation, morphometrics, population dynamics, genetics and conservation biology of *Trichosurus caninus*. In this paper, we outline the effects of sedation with tiletamine/ zolazepam on *T caninus*, physiological responses under sedation and observed side-effects of the drug combination.

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Materials and Methods

A total of 32 *T caninus* was captured at Cambarville (37°34'S, 145°53'E) in central Victoria during December 1993. Animals were trapped using large wire cage traps baited with apple. After capture, animals were transferred to a bag and then sedated with tiletamine/ zolazepam‡ in a 1:1 combination by weight by intramuscular injection into either the gluteal muscles or the biceps femoris and vastus lateralis. Sedation was required to facilitate the completion of a physical examination and the collection of blood samples. The total dose of drug administered and the induction time were recorded. The induction time was defined as the interval (in minutes) from the administration of tiletamine/zolazepam until the animal was in lateral recumbency. While under sedation, animals were sexed, weighed, tattooed for future identification, and a blood sample was collected from the jugular vein for haematological and serum biochemical analysis (Viggers K and Lindenmayer DB, unpublished). The pouch of each female was checked to determine the animal's reproductive status. Heart rate, respiratory rate and rectal temperature were recorded about 5 to 8 min after the administration by injection of the sedative to investigate the effects of the drug on these measures. Any side-effects of the sedation also were noted. After collection of blood and removal of ectoparasites, each animal was placed in a bag for recovery. The time to full recovery was not recorded.

Pouch young were removed and weighed only if they were unattached to the teat. Otherwise the young were carefully exteriorised from the pouch to record head length, tail length and crown to rump length while remaining attached to the teat and then immediately replaced in the pouch to avoid drying of the skin or hypothermia. A small number of advanced young, which were riding on the backs of

‡ Zoletil®, Virbac (Australia) Pty Ltd, Peakhurst, NSW

their mothers, were sedated for blood collection for future genetic analysis. For these animals, the dose rate of tiletamine/zolazepam was 10 to 15 mg/kg and the total dose administered was calculated on the basis of body weight.

Results

Rapid and safe sedation of *T caninus* using tiletamine/zolazepam was achieved in all animals and no deaths occurred. A standard dose of 50 to 60 mg was given initially to all animals and the induction times recorded are outlined below. Hypersalivation and licking movements commenced in all animals within one to two min of administration of the drug combination. This was followed by a progression of relaxation and deepening sedation until the animal was in sternal recumbency, and then, lateral recumbency.

The mean body weight of *T caninus* at Cambarville was 3.1 kg (± 0.4 kg). No significant difference in body weight was detected between males and females. The induction time varied from 2 to 6 min ($\bar{x}$ = 4 min ± 0.9 min). The mean dose rate was 19 mg/kg (± 3.2 mg/kg), which corresponded to a mean total dose of 59 mg (± 4.7 mg). There was no relationship between induction time and total dose administered or body weight of the animal. In addition, there was no significant effect ($P < 0.05$) of dose rate on heart rate, respiratory rate or body temperature.

The depth of sedation varied among individual animals, and if inadequate, additional smaller doses of tiletamine/zolazepam were given. Sedation was better in those animals given only one dose. A few animals began to recover 5 to 10 min before completion of handling procedures but they were easily restrained manually until all procedures were completed. Time to full recovery was not recorded, but it was estimated to range between 3 to 6 hours. Some animals commenced eating the apple provided in the recovery bag within 30 to 40 min of administration of tiletamine/zolazepam. As the swallowing reflex remained intact in all animals throughout sedation, and hypersalivation generally had ceased by the completion of handling procedures, we considered that it was safe to allow access to food during recovery.

Sedation in one animal was accompanied by excessive salivation commencing within 5 min of administration of the drug combination, followed by cyanosis and the onset of dyspnoea. Auscultation of the thoracic, tracheal and pharyngeal regions revealed harsh crackling and rustling sounds associated with the presence of fluid in the upper and lower respiratory tracts. The animal was placed in sternal recumbency with the neck extended to assist clearance of the airways and to facilitate breathing. Recovery to consciousness was prolonged in this case (60 min). The respiration appeared to return to normal within four hours, although it was not possible to repeat the thoracic auscultation after recovery due to the temperament of the animal.

The administration of tiletamine/zolazepam to females with pouch young that were attached to a nipple did not cause sedation in the offspring. Some females exhibited a reduced ability to hold the pouch closed during sedation. These animals were replaced in the recovery bag only after sufficient muscle tone was regained to ensure that the young would not be lost from the pouch.

Discussion

Wild *T caninus* are difficult to handle without sedation. Tiletamine/zolazepam provides rapid, safe, effective sedation of *T caninus* in the field, with few apparent side-effects. There appears to be a wide safety margin in the dose rate of the drug combination for this species. The average dose rate of 19 mg/kg (range 13 to 26 mg/kg) was higher than that recommended for other species including domestic animals (9 to 13 mg/kg) (Booth 1988) and macropods (10 mg/kg). The slightly longer induction time in males may be related to them being more aggressive and excitable than females.

For most animals, the standard total dose of 50 to 60 mg provided adequate sedation for blood sampling, ear tattooing, pouch checking, morphometric measurement and the collection of ectoparasites. A lower total dose may be administered for less time-consuming procedures. Full recovery after the administration of tiletamine/zolazepam may take several hours and animals should not be released before this, as sedation may affect their ability to climb or escape from predators.

Only one adverse reaction was recorded in our study. The onset of cyanosis, dyspnoea and excessive salivation in this animal might have been due to tiletamine/zolazepam. Alternatively, an underlying pathological process might have predisposed the animal to these unusual effects (Short 1987). Hypersalivation may be reduced by the administration of atropine. However, as laryngeal and pharyngeal reflexes remain intact, the control of salivation with atropine may not be necessary. In addition, tachycardia may result from the administration of tiletamine/zolazepam and this may be exacerbated by the positive chronotropic effects of atropine (Adams 1982).

Hunt *et al* (1988) found that benzodiazepine tranquilisers may induce hyperphagia in animals. This may explain why several *T caninus* commenced eating within 30 to 40 min of the administration of the sedative and before full recovery.

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Variation in Hematological and Serum Biochemical Values of the Mountain Brushtail Possum, *Trichosurus caninus* Ogilby (Marsupialia: Phalangeridae)

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ABSTRACT: Hematological and serum biochemical values were determined in a wild population of the mountain brushtail possum (*Trichosurus caninus*) at Cambarville, central Victoria, southeastern Australia. Animals were sampled during two-week trapping periods in June, September, and December 1992, and April 1993. Values for hemoglobin, red cell count and hematocrit were significantly higher in males than females. Total protein and mean corpuscular volume (MCV) were significantly higher in female *T. caninus*. Significant seasonal variations were detected for total bilirubin, alkaline phosphatase, total protein, albumin, urea, absolute eosinophils, MCV, sodium, potassium, and phosphate.

Key words: Hematology, serum biochemistry, mountain brushtail possums, *Trichosurus caninus*.

Hematological and serum biochemical analyses may be used to detect organ dysfunction and disease (Whittington and Grant, 1983). This type of information may make it possible to characterize changes in the health of a natural population in terms of the physiological and pathological responses of individual animals (Bradley, 1990). Despite the potential uses of blood chemistry investigations, there have been few attempts to establish reference values for many Australian species (Canfield et al., 1989).

Our objective was to determine the variation in blood chemistry values arising from differences between the sexes, seasons, and female reproductive status of the mountain brushtail possum, *Trichosurus caninus* Ogilby, at Cambarville in central Victoria, southeastern Australia. An additional objective was to establish reference values for the species.

Trichosurus caninus is a herbivorous, nocturnal, arboreal, phalangerid marsupial which inhabits tall wet forests and rainforests in eastern Australia (Owen and Thomson, 1965; How, 1972). Some hematological values in a wild population of *T. caninus* in northeastern New South Wales were reported by Barnett et al. (1979a, b), however serum biochemical values were not determined by these authors.

A population of *T. caninus* was trapped in a 15-ha area at Cambarville (37°34′S, 145°53′E) in central Victoria. The region has been described by Seebeck et al. (1984). The possums were trapped during 2-wk periods in June, September, and December 1992, and April 1993. Animals were sedated by intramuscular injection of Zoletil® (15 mg/kg) (tiletamine hydrochloride and zolazepam hydrochloride in a 1:1 ratio by weight) (Virbac, Sydney, Australia) to facilitate physical examination and the collection of blood samples. In addition, animals were weighed, measured and tattooed for future identification. The pouch of each female was checked to determine reproductive status.

From each animal, 1ml of blood was collected from the jugular vein using a 3ml syringe and 25 g needle and transferred into a blood tube containing ethylene diamine tetraacetic acid (EDTA). Samples were held in a refrigerator at 4 C and then forwarded on ice to Dorevitch Pathology of Camberwell, Victoria. Hematological analyses were completed within 16 hr of blood collection using a Sysmex NE-8000 Automated Hematological Analyser (JOA Medical Electronics Co Ltd., Kobe, Japan). Thin smears were made from the EDTA blood within 1 hour of collection, fixed in methanol and stained with Giem-

TABLE 1. Reference red blood cell values, red cell indices, total white cell and differential cell counts for the mountain brushtail possum, *Trichosurus caninus*, (n = 80) at Cambarville, Victoria, June 1992–April 1993.

Parameter	Median	5th–95th percentiles
Hemoglobin (g/dl)	12.2	10.5–14.1
Packed cell volume (%)	36.0	29.9–42.3
Red cell count ($\times 10^{12}$/l)	4.78	3.99–5.92
Mean corpuscular hemoglobin concentration (g/dl)	34	32–35
Mean corpuscular volume (fl)	74.3	68.1–80.0
Mean corpuscular hemoglobin (pg)	25.5	23.2–26.7
White cell count ($\times 10^{9}$/l)	4.2	2.1–6.8[a]
Neutrophils	2.1[a] (53%)[b]	0.5–4.8[a] (18–79%)[b]
Lymphocytes	1.6[a] (40%)[b]	0.6–3.4[a] (18–75%)[b]
Monocytes	0.1[a] (2%)[b]	0–0.5[a] (0–7%)[b]
Eosinophils	0.1[a] (1%)[b]	0–0.5[a] (0–9%)[b]
Basophils	0[a] (<0.01)[b]	0

[a] Absolute counts: units = $\times 10^{9}$/l.
[b] Percent of total white blood cells.

sa. These stained smears were used to complete differential white cell counts and to examine for the presence of blood parasites.

We collected 1.5 to 2 ml of blood from each animal into a plain serum tube. After clotting had taken place (2 to 3 hr), the blood was centrifuged to separate the serum from the clot. The resulting serum was stored and transported as for EDTA blood. Biochemistry analyses were performed within 16 hr of blood collection by Dorevitch Pathology using a Kodak Ektachem E 700 Automated Biochemistry Analyser (Johnson and Johnson Clinical Diagnostics, Rochester, New York, USA).

Results of hematological and serum biochemical analyses were evaluated for significant differences between the sexes, seasons, and the reproductive status of females. Seasons were defined as winter (June through August), spring (September through November), summer (December through February), and autumn (March through May). As some *T. caninus* were captured more than once over the four seasons, data were unbalanced and included both between and within animal components. Restricted maximum likelihood (Robinson, 1991) was used to estimate random effects for between animal and within animal variability, as well as fixed effects for the factors of interest, including sex, season, and reproductive status. A Wald test (χ^2) was applied to test the significance of the fixed effects (Genstat 5 Committee, 1993). Reference blood values for *T. caninus* at Cambarville were determined by combining all data from all animals, including repeat samples within animals, to determine the median, 5th and 95th percentile values for each parameter.

We captured 33 adult *T. caninus*, 20 females and 13 males, at Cambarville between June 1992 and April 1993. Most animals were trapped at least twice, for a total of 80 captures. Hematological and serum biochemical reference values were established using data from all *T. caninus* captured (Tables 1 and 2). No significant differences between lactating and non-lactating females were found for any blood values.

Significant differences between male and female *T. caninus* were detected for several red blood cell values (Table 3). Hemoglobin, red cell count (RCC) and hematocrit (PCV) were higher in males than females. Mean corpuscular volume and mean corpuscular hemoglobin were greater in females than males. Higher values for hemoglobin, RCC, and PCV in males also

TABLE 2. Reference serum biochemical values for *Trichosurus caninus* ($n = 80$) at Cambarville, Victoria, June 1992 to April 1993.

Parameter	Median	5th–95th percentiles
Urea (mmol/l)	9.5	5.8–15.8
Creatinine (mmol/l)	0.08	0.05–0.10
Total bilirubin (mmol/l)	7	2–18
Gamma-glutamyltransferase (IU/l)	19	13–39
Alanine aminotransferase (IU/l)	33	13–73
Alkaline phosphatase (IU/l)	1,508	838–2,977
Total protein (g/l)	62	55–647
Albumin (g/l)	38	34–42
Globulin (g/l)	24	19–29
Aspartate aminotransferase (IU/l)	123	79–240
Creatine phosphokinase (IU/l)	418	103–1,676
Lactate dehydrogenase (IU/l)	73	15–627
Glucose (mmol/l)	6.8	5.7–9.0
Amylase (IU/l)	472	242–672
Cholesterol (mmol/l)	2.13	1.52–3.64
Triglycerides (mmol/l)	0.80	0.47–1.61
Sodium (mmol/l)	144	141–148
Potassium (mmol/l)	3.5	2.7–4.9
Chloride (mmol/l)	101	94–108
Bicarbonate (mmol/l)	30	24–34
Calcium (mmol/l)	2.36	2.12–2.53
Phosphate (mmol/l)	1.3	0.8–2.2

have been recorded in *T. caninus* and *T. vulpecula* at Clouds Creek in north-eastern New South Wales (Barnett et al., 1979a) and in *T. vulpecula* in good condition in Victoria (Presidente and Correa, 1981).

No significant differences in the total and differential white cell counts were found between the sexes or the seasons, except for the absolute eosinophil count, which was highest in autumn ($P < 0.001$). Parasitism is a common cause of elevation of eosinophils in the peripheral circulation (Bush, 1991).

Neutrophils were the predominant white blood cell in the peripheral blood of *T. caninus*. This may be a normal feature of this species or it may be the result of a physiological response to handling. Differential and total white cell counts were not performed for *T. caninus* in northeastern New South Wales (Barnett et al., 1979a), hence comparison between the two studies is not possible. Total white cell counts in *T. caninus* were lower than those reported from *T. vulpecula* (Presidente and Correa, 1981). These authors also reported a neutrophilia in conjunction with a relative lymphopenia and eosinopenia in *T. vulpecula* in poor condition, and attributed these changes to stress.

Total protein levels were significantly higher in female *T. caninus* ($P < 0.02$). However, differences in other blood measures could not be attributed to the sex of animals. Seasonal effects were detected for several biochemistry parameters (Table 4). Levels of urea were highest in spring and serum protein levels were highest in summer. Significantly higher serum protein levels in summer also were reported by Barnett et al. (1979a) for *T. caninus* in northeastern New South Wales. Presidente and Correa (1981) did not find significant differences by sex in serum protein levels in *T. vulpecula*; values for urea in

TABLE 3. Variation between sexes in the hematology of *T. caninus* at Cambarville, June 1992 to April 1993. Data were derived from 33 individuals (13 males, 20 females) and include repeat measures for animals captured more than once, giving a total of 80 measures for each parameter.

Parameter	Males mean (SE)	Females mean (SE)	*P* value
Hemoglobin (g/dl)	12.7 (0.15)	11.9 (0.2)	0.008
Packed cell volume (%)	37.6 (0.7)	34.9 (0.6)	0.006
Red cell count ($\times 10^{12}$/l)	5.1 (0.1)	4.6 (0.1)	0.007
Mean corpuscular volume (fl)	72.2 (0.6)	75.5 (0.5)	0.001
Mean corpuscular hemoglobin (pg)	24.6 (0.2)	25.9 (0.2)	0.001

TABLE 4. Seasonal variation in serum biochemistry values for *T. caninus* at Cambarville (June 1992–April 1993)

Parameter	Winter (n = 18) mean (SE)	Spring (n = 21) mean (SE)	Summer (n = 20) mean (SE)	Autumn (n = 20) mean (SE)	*P* value
Urea (mmol/l)	9.2 (0.6)	12.2 (0.5)	9.3 (0.5)	8.7 (0.5)	0.001
Total bilirubin (mmol/l)	11 (1.5)	12 (1.0)	5 (0.5)	5 (0.1)	0.001
Alkaline phosphatase (IU/l)	1,316 (99)	1,809 (104)	2,135 (107)	1,120 (105)	0.001
Total protein (g/l)	61.8 (0.8)	59.0 (0.1)	62.6 (0.7)	61.2 (0.7)	0.002
Albumin (g/l)	38.7 (0.6)	35.6 (0.5)	37.6 (0.5)	38.4 (0.5)	0.001
Lactate dehydrogenase (IU/l)	481 (84)	187 (27)	96 (14)	85 (25)	0.001
Glucose (mmol/l)	7.0 (0.2)	6.5 (0.2)	6.5 (0.2)	7.1 (0.2)	0.006
Sodium (mmol/l)	147 (0.5)	143 (0.5)	144 (0.5)	145 (0.5)	0.001
Potassium (mmol/l)	4.0 (0.1)	3.5 (0.1)	3.5 (0.1)	3.4 (0.1)	0.001
Phosphate (mmol/l)	1.5 (0.1)	1.6 (0.1)	1.1 (0.1)	1.2 (0.1)	0.001

this species were lower than our findings for *T. caninus*. Elevated dietary protein may be a major factor influencing variation in serum urea levels (Seal et al., 1975; McCue and O'Farrell, 1992). Seebeck et al. (1984) evaluated the diet of *T. caninus* at Cambarville, but these authors did not investigate the nutrient composition of dietary items.

Total bilirubin levels were lower in summer and autumn than winter and spring, while alkaline phosphatase (ALP) was highest in summer. Values for ALP generally were high in *T. caninus* (mean 1631 IU/l; range 777 to 3435) when compared with those from other marsupials (Whittington and Grant, 1983; Canfield et al., 1989). The ALP isozyme in bone may cause an elevation of serum ALP in young growing animals (Seal et al., 1975; Smith and Rongstad, 1980). However, as only adult *T. caninus* were examined in this study, this is unlikely to be a cause of the high levels of ALP that were recorded. These values may be normal for *T. caninus*, as animals appeared to be healthy and there was no concurrent increase in other hepatic enzymes, which may be expected if there was any associated liver pathology (Bush, 1991).

Seasonal variation was detected in serum sodium, potassium and phosphate levels. Serum sodium levels in *T. caninus* were highest in winter. Increased serum sodium levels may result from excessive water loss, decreased water intake, or increased sodium intake (Bush, 1991), which may be related to seasonal dietary variation. Elevation of serum sodium levels in summer in the red kangaroo (*Macropus rufus*) and the euro (*Macropus robustus*) was attributed to variation in the dietary intake of sodium from different species of grass (Dawson and Denny, 1969). Serum potassium levels in *T. caninus* were highest in winter, which may be associated with reduced dietary sodium intake (Bush, 1991). Hence, the seasonal differences in sodium and potassium levels in *T. caninus* at Cambarville may be associated with fluctuating sodium intake due to dietary variation. Studies by Seebeck et al. (1984) and Claridge and Lindenmayer (1993), investigating the diet of *T. caninus* at this site detected significant seasonal variation in the intake of several dietary items, including fungi and the foliage of plants from the forest understorey and ground layers. Phosphate levels were higher in winter and spring than the other seasons ($P < 0.03$). Increased serum phosphates may be attributable to high phosphorus levels in the diet (Bush, 1991).

The serum concentrations of sodium, potassium, and chloride in *T. caninus* were similar to those of *T. vulpecula* in good condition. Both calcium and phosphate levels in *T. caninus* were lower than *T. vul-*

pecula in both good and poor condition (Presidente and Correa, 1981).

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